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A SEX-LIMITED COLOR IN AYRSHIRE CATTLE[1]

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TYPES OF INHERITANCE AS RELATED TO SEX

Two general types of inheritance as related to sex exist, aside from the ordinary secondary sex characters. Sex-linked inheritance depends on the great mass of hereditary factors that have been shown to be linked in transmission to the sex-determining factors; while sex-limited factors follow the simple Mendelian scheme of inheritance, but show a reversal of dominance in the two sexes. Frequently these two latter terms are used synonymously, but since there is a distinction between the two classes of transmission, and since the term "sex linked" is so much more descriptive of the hereditary phenomena to which it has been applied than is the term "sex limited," the foregoing terminology is used.

HISTORICAL REVIEW

The classical case of sex-limited inheritance was reported by Wood (7), who made reciprocal crosses of the Dorset sheep, a breed horned in both sexes, with the Suffolk, a breed polled in both sexes. All F_1 individuals were the same, so far as the type of cross was concerned, the males being horned and the females polled. In the F_2 generation the fact that dominance differed in the two sexes resulted in three males being horned to one being polled, and three females being polled to one being horned.

Similarly in 1912 the writer reported a pair of rudimentary teats in swine, located on the lower part of the scrotum of the male and on the inner thighs of the female, behind the inguinal pair, which presented the same phenomenon in transmission, the character being dominant in the male and recessive in the female.

Gerould (2)[2] reported in 1911 on the inheritance of yellow and white in the common clover butterfly (*Colias philodice*). White is dominant to

[1] Paper No. 3 from the Laboratory of Animal Technology, Kansas Agricultural Experiment Station.
[2] Reference is made by number to "Literature cited," p. 147.

yellow in the female, but it is recessive in the male. Something lethal seems to be connected with homozygosis for white; hence, white as a somatic character appears only in the female. The yellow female is YY, the white female YW. Males are either YY or YW, but are always yellow.

Jacobson (3) made some observations on *Papilio mennon* L., which were studied from a Mendelian standpoint by De Meijere (5) in 1910. There are three varieties of females in this species known as Achates, Agenor, and Laomedon, respectively, in the order of their dominance. The males corresponding to these three forms are all alike, although each of the female patterns may be carried in a recessive manner. Furthermore, De Meijere believes that the female carries the male pattern homozygously; but, owing to the reversal of dominance, the male character never becomes somatic. The Laomedon probably represents the female expression of the male condition. The principal difference between this and the previous cases is that the changes in dominance affect the homozygotes as well as the heterozygotes.

AYRSHIRE BLACK-AND-WHITE

A case which seems to fall under this general sex-limited group is found in the inheritance of black-and-white as alternative to red-and-white in Ayrshire cattle. While the general breed color is red-and-white, black-and-white animals have been known for some time, as shown by Kuhlman (4). Practically no attention has been paid to the mode of inheritance of this color, since in America it has been considered undesirable and selection against it has been practiced. It is difficult to state whether the black is due to a true black pigment or whether it is simply a very dense red. Under the microscope typically black granules seem to be present, but no chemical solutions of the pigments have yet been attempted.

SOURCE OF THE DATA [1]

The Ayrshire herd bull at the Kansas Experiment Station, Melrose Good Gift, is a very deep mahogany-and-white; in fact, the black-and-white previously referred to. It is through the study of his ancestry and breeding performance, the ancestry and breeding performances of the cows in the herd, including the black-and-white animals, and the records of some of the former herd bulls that the present data were secured. In all, 63 individuals were included. Much larger numbers might have been obtained by adding the progeny of red-and-white males and females to the table; but since they demonstrated no facts different from those here included, their records are not presented.

[1] Acknowledgments are hereby made to Prof. O. E. Reed, of the Department of Dairy Husbandry, Kansas Experiment Station, for facilities extended in obtaining the data.

PROGENY OF MELROSE GOOD GIFT FROM RED-AND-WHITE COWS

Fifteen red-and-white cows in the herd were mated to Melrose Good Gift to produce 20 calves, of which 10 were black-and-white bulls and 10 were red-and-white heifers. All of the bulls were as red as the heifers at birth, but at 2 to 4 months of age the blackish tinge began to develop, and within 4 months the youngsters became distinctly black-and-white. The heterozygous male progeny of Melrose Good Gift differed from the homozygous male progeny in that the black tinge developed more slowly and also became much less intense on maturity. While in the mature homozygous bull the black is very distinct throughout the pigmented areas, in the mature heterozygous bull the black may appear only as a streaked border where the pigmented spots adjoin the white, or at the limbs, muzzle, ears, and tail. The main portions of the colored parts of the animal are usually a very dark red which blends gradually, although in a particulate manner, into the blacker borders. The heterozygous heifers are red-and-white, and while occasional dark hairs are found, no regular means whereby the heterozygous red-and-white females could be distinguished from the homozygous red-and-white females was discovered. It should be further noted that the black color of the homozygous female is by no means as intense as that of the male, although the black is indisputably present.

HETEROZYGOUS BLACK BULLS TO HOMOZYGOUS RED COWS

Johanna Croft King, College Marquis, Sir Croft of Spring City, Woolford's Good Gift, and Lessnessock Oyama's Good Gift were bulls which by their breeding performance and somatic description must have been heterozygous for the black factor. The last two bulls were found in the pedigree of Melrose Good Gift, while the first three were used at one time or another at the college as herd bulls. Records of these in matings to homozygous red-and-white cows were available for all except Woolford's Good Gift, and the result showed four red-and-white heifers, four black-and-white bulls, and 5 red-and-white bulls. This is the most probable distribution of colors in both the males and females and is perfectly in alignment with the interpretation of the method of inheritance as given.

The reciprocal cross of red-and-white bulls to black-and-white cows gave two black bulls to one red bull and two white heifers, also the most probable expectation.

BLACK-AND-WHITE COWS MATED TO RED-AND-WHITE BULLS

Only three calves were available from this type of mating, all red-and-white daughters of Bangora, the original black-and-white cow in the herd. While the numbers are too small to be conclusive, yet they conform to the expectation.

RESULTS OF THE DIFFERENT CROSSES

If the factor for the black-and-white color is represented by B, the hereditary constitutions are as follows: BB is always black-and-white; bb is always red-and-white; Bb is always black-and-white in the male and red-and-white in the female. All of the nine possible matings were discovered, as shown in Table I.

TABLE I.—*Results of nine possible matings of Ayrshire cattle*

Sires.	Dams.	Male offspring.		Female offspring.	
		Black-and-white.	Red-and-white.	Black-and-white.	Red-and-white.
BB	BB	1	0	3	0
BB	Bb	0	0	0	1
BB	bb	10	0	0	10
Bb	BB	3	0	2	1
Bb	Bb	1	0	1	0
Bb	bb	4	5	0	4
bb	BB	0	0	0	3
bb	Bb	2	1	0	2
bb	bb	0	7	0	9
Total		21	13	6	30
Expected		20.75	13.25	5.25	30.75

The expectations here presented are based on the most probable result of each of the matings, considered on an individual basis with reference to the number of animals produced by each type of mating, but without figuring the proportions of the sexes as equal. From these data it would appear that the black-and-white color of Ayrshire cattle behaves in an ordinary sex-limited manner similar to the horns in sheep as discussed by Wood (7) and the rudimentary mammæ in swine as reported by the writer (6).

DISCUSSION

Arkell and Davenport (1) have reported on the inheritance of horns in sheep and have attempted to bring it under the ordinary sex-linked scheme of inheritance by an ingenious system of inhibitors and horn factors. Such an explanation was doubtless justified when horns in sheep were the only character known in which the reversal of dominance in the two sexes existed, but now that at least two other characters are known in which an exactly similar system of inheritance occurs, it seems unnecessary to assume the complexities hypothesized by these investigators. Instead, the much simpler and probably more perfectly descriptive explanation adopted by Wood (7) in his original paper seems more logical.

COLOR RECORD OF PROGENY IN AYRSHIRE CATTLE

The following record presents the data considered in this paper. The term "red" refers to red-and-white and the term "black" refers to black-and-white. The hereditary constitution assigned the breeding animals retained in the herd or found in the pedigrees of animals in the herd is also given.

Johanna Croft King, Bb (described as dark).
- Sir Croft of Spring City, Bb (black).
- Johanna of Juneau, bb (red).

College Marquis, Bb (described as dark).
- Marquis of Woodruff, bb (red).
- Maggie of Woodruff, Bb (red).

Woolford's Good Gift, Bb (described as mahogany).
- Lessnessock Oyama's Good Gift, Bb (described as dark).
- Pearl 3d of Woolford, bb (red cow).

Melrose Good Gift, BB (black-and-white).
- Woolford's Good Gift Bb.
- Florence Melrose, Bb (red cow).

College Maud, bb (red).....
- Marquis of Woodruff, bb (red).
- Star of Hillview, Bb (red).

Bangora, BB (black).....
- White Prince, Bb (described as mahogany in pigmented areas).
- Star of Hillview, Bb (red).

College Marquis 2d, bb (red)
College Marquis 3d, bb (red).....
(See progeny of College Maud.)
- College Marquis, Bb (dark).
- College Maud, bb (red).

Progeny of College Maud 31350 (red), bb:
- One red heifer by unknown red bull, bb.
- One red heifer by College Marquis, Bb.
- Three red bulls by College Marquis, Bb.
- One red heifer by Johanna Croft King, Bb.
- One red heifer by Sir Croft of Spring City, Bb.
- One red heifer by Melrose Good Gift, BB.

Progeny of College Maud 2d (red), bb (daughter of College Maud by College Marquis):
- One red heifer by College Marquis, Bb.

Progeny of College Maud 2d's heifer (red), bb (daughter of College Maud 2d by College Marquis):
- One black bull by Sir Croft of Spring City, Bb.
- One red bull by College Marquis 3d, bb.
- One black bull by Melrose Good Gift, BB.
- One red heifer by Melrose Good Gift, BB.

Progeny of Kansas Croft Maud (red), Bb (daughter of College Maud by Sir Croft of Spring City):
- One red heifer by Melrose Good Gift, BB.
- One black bull by Cavalier's College Master, bb.

Progeny of Johanna Croft Maud (red), bb (daughter of College Maud by Johanna Croft King):
- One red heifer by Melrose Good Gift, BB.

Progeny of Georgie Em 25749 (red), bb:
One red heifer by Sir Croft of Spring City, Bb.
One red heifer by College Marquis 3d, bb.
One black bull by Melrose Good Gift, BB.
One red heifer by Melrose Good Gift, BB.
One red heifer by College Marquis 2d, bb.

Progeny of Georgie Croft (red), bb (daughter of Georgie Em by Sir Croft of Spring City):
Three black bulls by Melrose Good Gift, BB.

Progeny of Marquis Em (red), bb (daughter of Georgie Em by College Marquis 3d):
One red heifer by Melrose Good Gift, BB.
One black bull by Melrose Good Gift, BB.

Progeny of Johanna of Juneau 26290 (red), bb:
One black bull by Sir Croft of Spring City, Bb.
One red heifer by College Marquis 3d, bb.
Twins (one black bull and one red heifer) by Melrose Good Gift, BB.
One red heifer by College Marquis 2d, bb.

Progeny of Elizabeth of Juneau 26292 (red), bb:
One red bull by Sir Croft of Spring City, Bb.
One red bull by College Marquis 3d, bb.
Two black bulls by Melrose Good Gift, BB.

Progeny of Rose of Oakdale 26291 (red), bb:
Two red bulls by College Marquis 2d, bb.
One red bull by College Marquis 3d, bb.
One red heifer by Melrose Good Gift, BB.
One red heifer by Cavalier's College Master, bb.

Progeny of Rosa Lee Melrose (red), bb (daughter of Rose of Oakdale by Melrose Good Gift, BB):
One red bull by Cavalier's College Master, bb.

Progeny of Canary Belle 25748 (red), bb:
One red bull by Sir Croft of Spring City, Bb.
One red bull by College Marquis 3d, bb.
One red heifer by Melrose Good Gift, BB.
One black bull by Melrose Good Gift, BB.
One red heifer by Cavalier's College Master, bb.

Progeny of Melrose Canary Belle, (red), Bb (daughter of Canary Belle by Melrose Good Gift, BB):
One red heifer by Cavalier's College Master, bb.

Progeny of Fearnot of Oakdale 26289 (red), bb:
One black bull by Sir Croft of Spring City, Bb.
One red heifer by College Marquis 3d, bb.
One red heifer by Melrose Good Gift, BB.
One red bull by College Marquis 2d, bb.

Progeny of Lady Marquis Fearnot (red), bb (daughter of Fearnot of Oakdale by College Marquis 3d):
One red heifer by Melrose Good Gift, BB.

Progeny of Bangora 29700 (black), BB:
One red heifer by Marquis of Woodruff, bb.
One red heifer by College Marquis, Bb.
Two black heifers by College Marquis, Bb.
One black bull by Sir Croft of Spring City, Bb.
One black bull by Johanna Croft King, Bb.
One black heifer by Melrose Good Gift, BB.
One black bull by Melrose Good Gift, BB.
One red heifer by Cavalier's College Master, bb.

Progeny of Bangora 2d (black), BB (daughter of Bangora by College Marquis):
One black bull by Johanna Croft King, Bb.
Two black heifers by Melrose Good Gift, BB.

Progeny of Bangora's Melrose (black), BB (daughter of Bangora by Melrose Good Gift, BB):
One red heifer by Cavalier's College Master, bb.

CONCLUSIONS

(1) Black-and-white color is a simple allelomorph of red-and-white color in Ayrshire cattle.

(2) In the male the black-and-white character is dominant and in the female the red-and-white character is dominant.

(3) Males heterozygous for the two characters are black-and-white, while females heterozygous for the two characters are red-and-white.

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WATERMELON STEM-END ROT

[PRELIMINARY PAPER]

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During the last few years in certain parts of the United States shippers have been seriously troubled by a decay which attacks watermelons (*Citrullus vulgaris*) in transit and may sometimes destroy or render unsalable a large percentage of a shipment before it reaches its destination. Owing to this fact, in the season of 1915 the Department of Agriculture began a careful investigation of shipping conditions, in the course of which the present writer had an opportunity to make a laboratory study of some decayed material.

This material was taken from a shipment received in Washington, D. C., on July 24, 1915. The shipment consisted of five carloads of approximately 900 watermelons each, no one car of which yielded more than 300 salable melons, owing to the prevalence among them of the disease. The decayed watermelons were distributed through the car entirely without reference to position, a fact which made it seem manifestly impossible that the trouble could have originated from mechanical or chemical injury received from contact with the walls or the floor of the car.

This examination indicated, moreover, that, as has been reported in the case of other shipments, the injury of these watermelons had occurred in a very uniform manner. In its early stages the presence of the decay was indicated by a watery discoloration of the rind in an area closely surrounding and apparently extending from the stem. Beginning in this way there were all stages of decay up to those where about half or three-quarters of the melon were involved. In such cases the rind of this portion had become soft and wrinkled, so that in cross section it appeared much like that of the watermelons shown in the lower row of Plate XVII, figure 1. The meat below this part of the rind was slimy and blackened, while that at the opposite end of the melon remained sound, not having as yet become included in the decay. Owing to the warm, moist conditions at this season, the portion involved was covered by a gray or somewhat black mold, so that the origin of the trouble could not be readily ascertained.

An abundance of material being available at this time, an attempt was made to find out whether the injury was due to the action of some fungus, and, if this proved to be the case, to obtain the specific organism

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in pure culture. In endeavoring to obtain such cultures, the following procedure was adopted. Several watermelons were selected in which the decay was just beginning to be apparent. A razor was flamed; and with this, a funnel-shaped section, which included a portion of both diseased and healthy tissue, the two being separated by a more or less distinct line of demarcation, was cut from the melon. After the razor had been flamed again, the section was divided along the line of demarcation which distinguished the advancing edge of the decay, the plug being cut from the inside toward the outer surface. This gave access to a portion of the rind to which the fungus filaments were probably just advancing and which would be unlikely to contain concomitant forms. From this region, using a sterile platinum needle, small portions were removed from just below the surface and placed directly on synthetic agar in sterile Petri dishes. After two days, during which the plates were kept at a temperature of 27° C., an abundant mycelial growth of a gray color appeared in every instance. A number of transfers of the mycelium thus obtained were made to potato cylinders, and in all cases a fungus developed which possessed the characteristics of the genus Diplodia. In order to test the capacity of this organism for producing the decay, the pure culture was inoculated into a sound watermelon at three widely separated points, at each of which the characteristic rot was reproduced.

The direct connection between this fungus and the disease having been thus indicated, 16 healthy watermelons were obtained for more inoculations. They were bought at the wharf in Washington, D. C., and came from the Pyankatank River district in Virginia, a region free from the disease, so far as is known. It may be well to mention in this connection that the decay has usually been reported as occurring on the variety known as "Tom Watson." This is probably due to the fact that in the last few years this melon has been grown somewhat to the exclusion of other varieties. Of the melons chosen for inoculation, three were "Excel" melons; the remainder were of the "Tom Watson" variety.

These melons were placed on a table near a large window which was kept open the greater part of the day, and were protected from the direct light of the sun by a cardboard screen. For a period of nine days, during which time the melons were under observation, the average temperature was 26.5° C. Of these 16 watermelons, 8, two of which were of the "Excel" variety, were inoculated with the fungus, the cultures used in this case having been derived from the original subculture. This was accomplished by making with a sterile knife at a single point near the stem an incision, into which a bit of the growing fungus mycelium was introduced. A similar wound was made in the remaining 8 melons, including the third "Excel" variety, but no infectious matter was introduced. Within 36 hours the 8 inoculated melons began to show

signs of decay, while the 8 checks remained perfectly sound throughout the course of the experiment. There was no decay present on the inoculated melons except that which originated at the point of inoculation.

The decay is first noticeable as a somewhat circular discolored area surrounding and extending from the point of inoculation. On the watermelons observed in the laboratory this area gradually increased in size until at the end of six days about half of the melon was involved. At this time the advance of the decay seemed to become less rapid and the area which was first decayed began to show a blackening due to the formation of pycnidia by the fruiting fungus. This area spread daily, and at the close of nine days the stem end of the melon presented a withered, charred appearance. Plate XVII, figure 1, is a reproduction of a photograph of nine of these melons. The four in the upper row are checks; the five below were inoculated.

The fructification of the fungus may be briefly described as follows: Pycnidia separate or confluent, smooth or, under moist conditions, covered with loose olivaceous hyphæ, 180 to 250μ in diameter. Spores 24 to 30μ by 10 to 14μ, oval, uniseptate, dark brown. On the material taken from the watermelons inoculated in Washington no paraphyses could be detected. They are present, however, when the organism is grown upon potato cylinders, a fact which would tend to support the conclusions reached by Taubenhaus,[1] to whose work reference will be made in the following paragraph.

It has long been known that those members of the Sphaeropsideae which produce brown uniseptate spores are extremely variable. The distinctions between the genera Diplodia, Botryodiplodia, Chaetodiplodia, Lasiodiplodia, and Diplodiella have been based on slight structural variations in the pycnidia. The points of separation are the relation of the pycnidia to one another, whether scattered or cespitose; their relation to the host, whether subcutaneous, erumpent, or superficial; the presence or absence of bristles and of paraphyses. These are all characteristics which one might expect to vary somewhat with the characteristics or the condition of the host. This variation probably occurs; and for this reason there has been some uncertainty as to the proper position certain species should occupy in classification. *Botryodiplodia theobromae* Pat., which causes a dieback of *Hevea braziliensis* in Ceylon, southern India, and the Malay States, is an example; and in his account of this fungus Petch[2] remarks that—

> Among the names which are known to refer to this species are *Macrophoma vestita*, *Diplodia cacaoicola*, *Lasiodiplodia theobromae*, *Diplodia rapax*, and there are probably others. *Botryodiplodia theobromae* is its earliest name, as far as is known, but some prefer to call it *Lasiodiplodia theobromae*.

[1] Taubenhaus, J. J. The probable non-validity of the genera Botryodiplodia, Diplodiella, Chaetodiplodia, and Lasiodiplodia. In Amer. Jour. Bot., v. 2, no. 7, p. 324-331, pl. 12-14. 1915.

[2] Petch, Thomas. Physiology & Diseases of Hevea braziliensis . . . 268 p., 16 pl. London, 1911.

Taubenhaus, as a result of his inoculations upon sweet potato (*Ipomoea batatas*) with *Diplodia tubericola* E. and E., *Diplodia gossypii* Zim., *Diplodia natalensis* Pole Evans, and *Lasiodiplodia theobromae* (Pat.) Griff. and Maubl., suggests that the characteristics of the genus Diplodia be so extended that it may include all of the five genera.

This genus, although it is not thought to include forms which are absolute parasites, is nevertheless a source of serious trouble among some of our cultivated plants. The injury is usually confined to a fruit rot or to a dieback of the younger branches or shoots as in the Citrus disease prevalent in Florida and the Isle of Pines.[1] In both cases the fungus has been described as following an injury which has been previously inflicted either by mechanical means or as the result of the action of some other fungus. In the United States the more important crops which hitherto have been known to be affected are sweet potato, Citrus fruits, corn (*Zea mays*), and cotton (*Gossypium* spp.) In our Southern States the *Diplodia* injury is of considerable consequence in connection with these products. As one enters the Tropics the number of plants which are attacked increases. Among the list of hosts found here are *Citrus* spp., *Hevea* spp., *Theobroma cacao*, and *Thea* spp. In certain cases where the growing plant is attacked, the injury produced is sufficient to cause the death of the host, as is the case with *Diplodia vasinfecta* Petch, which causes an internal rootrot of tea.

Since the cotton, sweet-potato, and watermelon fields of the South are not widely separated, it is of some interest from the economic standpoint to know whether a species found on one host will grow equally well upon another. Plate XVII, figure 2, shows a watermelon nine days after it had been inoculated with a culture of *Diplodia tubericola* E. and E. obtained from Mr. L. L. Harter, of the Bureau of Plant Industry. The decay took the same course in this melon as has been described for the other inoculated material, which is shown in Plate XVII, figure 1. The pycnidia which were produced, however, retained the paraphyses.

While the *Diplodia* injury is apparently the cause of serious loss in the watermelon industry, there are other ways in which the crop suffers. Dr. W. A. Orton, Pathologist in Charge of Cotton and Truck Disease Investigations, Bureau of Plant Industry, who has made a careful study of shipping conditions, is inclined to believe that the injury is confined to certain districts. In other sections, anthracnose, due to *Colletotrichum lagenarium*, is the source of considerable trouble. To the losses thus caused by fungi must be added a small percentage of melons which have been damaged by rough treatment and by the use of cars which have been employed for the transportation of fertilizer or chemicals to the fields.

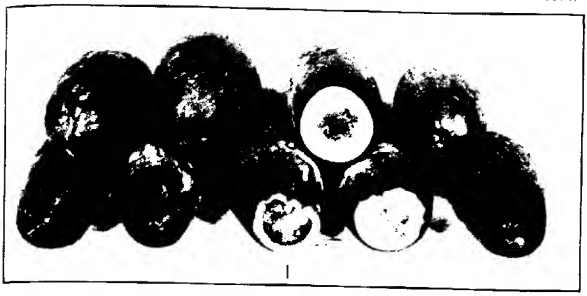
[1] Earle, F. S., and Rogers, J. M. Citrus pests and diseases at San Pedro in 1915. *In* San Pedro Citrus Path. Lab. 1st Ann. Rpt. 1915, p. 5-41, 19 fig. [1915.]

PLATE XVII

Watermelons, showing the effect of inoculation with species of Diplodia:

Fig. 1.—The upper four melons were held as checks; the lower five are melons nine days after having been inoculated with a culture of *Diplodia* sp. which had been isolated from a decaying watermelon obtained from a freight car at Washington, D. C.

Fig. 2.—A watermelon nine days after having been inoculated with a culture of *Diplodia tubericola* E. and E. .



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EFFECT OF PASTEURIZATION ON MOLD SPORES

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INTRODUCTION

Definite experiments to determine whether spores of the common saprophytic molds survive the temperatures used for the pasteurization of milk have not been reported. These spores are certainly present and are frequently abundant in ordinary market milk. Vague and general statements that such organisms do or do not survive are not uncommon, but are not supported by reference to actual work. To obtain such data studies were made with spores from pure cultures of a series of molds including several species of Penicillium, Aspergillus, and of the mucors, with, in some experiments, the addition of *Oidium* (*Oospora*) *lactis* and one strain of Fusarium. These sets of experiments were made to test, as carefully as laboratory conditions would permit, the temperatures used in pasteurization by the "holder" process, those used in the "flash" process, and the effects of dry heat.

EXPERIMENTS WITH THE HOLDER PROCESS OF PASTEURIZATION

Bacteriological studies of milk treated by the holder process have fixed the temperatures between 140° and 145° F. (60° to 62.8° C.), maintained for 30 minutes, as the minimum heating for the destruction of pathogenic organisms which may be found in milk. Although certain bacteria survive this heating it has been found that milk so treated is free from the ordinary disease-producing organisms, safe for consumption, unchanged in taste, and low enough in acid organisms to be handled without souring too quickly.

To study the effect of this process of pasteurization on mold spores, conidia from pure cultures of molds were first transferred to tubes of sterile water to obtain a suspension of spores. Transfers from such a suspension reduce the danger of such spores being blown by air currents into the cotton plugs and upon the walls of the test tubes used, where they might escape the full temperature applied to the milk. In the first series the inoculations were made by transferring 1 c. c. of this suspension in sterile pipettes into duplicate tubes of sterile milk. In a later series a platinum loop was used, since the tendency of the conidia to float thickly upon the surface of the water made this a quick and effective method of handling them. For most species it was thus possible to transfer spores enough to make a visible film over a part of the surface of the milk. None

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of the species used produced visible growth except upon or near the surface of the milk. Observations of growth must include, therefore, the surface of the milk and especially the glass from the surface of the milk upward for a few millimeters, since most molds begin to grow first upon the glass. When no spores occurred upon the glass a free-floating colony in one case escaped observation until it fruited.

The inoculated milk tubes, with the exception of the control tubes, were heated in a water bath in which the water was agitated and the temperature of the milk was recorded in a control tube by a thermometer placed in the milk. The temperature in the tubes was not allowed to vary more than half a degree in either direction. The results of the experiments with the holder process are shown in Table I. In preparing this table the records of the checks, or unheated tubes, of successive experiments were found sufficiently uniform to permit them to be averaged and appear but once. Experimental tubes were made in duplicate; and when the results were not reasonably harmonious the work was repeated. Table I summarizes the tabulated data from a series of experiments extending over a period of several months.

TABLE I.—*Comparative effect of heating mold spores in milk to temperatures of from 120° to 150° F. (48.9° to 65.6° C.) for 30 minutes* [1]

Name of mold.	Serial No.	Growth of spores when not heated (control).			Growth of spores when heated to temperature indicated and held for 30 minutes.																				
					120° F. (48.9° C.).			125° F. (51.7° C.).			130° F. (54.5° C.).			135° F. (57.2° C.).			140° F. (60.0° C.).			145° F. (62.8° C.).			150° F. (65.6° C.).		
		2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.
Aspergillus candidus	106	0.2	0.5	0.7	.....	.....	.....	0.1+	0.3	0.6	0.1+	0.3+	0.7	(?)	0.3+	0.6	(?)	(?)	0.4	(?)	0.0	0.0	.....	.....	.....
Aspergillus flavus (series)	108	.4	.6	.9	.....	.....	.....	.1—	.4	.9	(?)	.5	.8	(?)	.4—	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Do	ABC 3538	.5	.8	1.0	.....	.....	.....	.1—	.5	1.0	(?)	.5	.8	(?)	(?)	.5—	(?)	0.0	.0	(?)	.0	.0	.....	.....	.....
Do	Rg 136	.4	.9	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.1	.6	1.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Do	Sc 171	.4	.7	.9	.....	.....	.....	.....	.....	.....	(?)	.0	.0	(?)	.1y	.1	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Aspergillus fumigatus	118	.6	1.0	1.0	.....	.....	.....	.....	.....	.....	.1+	.9	1.0	.2	.9	1.0	.2+	.9	1.0	(?)	.3	1.0	(?)	0.2b	0.6b
Aspergillus glaucus	2705	.2	.4	.7	(?)	0.2	0.4	(?)	.2—	.3	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Do	3512	.2	.5	.8	0.2	.4	.7	(?)	.2	.5	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Do	3585. 21	.2	.5	.8	(?)	.4	.6	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Aspergillus nidulans	.....	.3	.9	1.0	.....	.....	.....	.2+	.8	1.0	.1—	.4	.7	(?)	.1y	.5	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Aspergillus niger (series)	111	.4	1.0	1.0	.....	.....	.....	.1+	.8	1.0	(?)	.3	1.0	(?)	.6	1.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Aspergillus niger, var. *altipes*	3534a	.4	.8	1.0	.....	.....	.....	.2+	.7	1.0	.1+	.7	1.0	(?)	(?)	.4	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Aspergillus niger (*cinnamomeus*)	3534b	.3	.7	1.0	.....	.....	.....	.1—	.4	.6	(?)	.3—	.8	(?)	(?)	.3	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Aspergillus niger (*fuscus*)	3534c	.3	.7	1.0	.....	.....	.....	.....	.4	.8	(?)	.4+	.8	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Aspergillus ochraceus	112	.3	.7	1.0	.....	.....	.....	(?)	.3	.7	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0
Aspergillus oryzae	113	.4	.6	.9	.....	.....	.....	(?)	.2—	.6	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0
Aspergillus repens	.....	.2	.5	.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.1	.5	.6	(?)	.0	.0	(?)	.0	.0	.8	.4	.6
Aspergillus wentii	116	.2	.7	.9	.....	.....	.....	(?)	.2—	.6	(?)	.0	.0	.....	.....	.....	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0
Aspergillus sp	Ra 42	.3	.6	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.2	.6	1.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Do	3522-39	.4	.8	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Do	3522-36	.2	.3	.5	.....	.....	.....	.1—	.25	.5	(?)	.2—	.3	(?)	.6	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Do	3556	.3	.4	.7	.....	.....	.....	.1	.2	.7	(?)	.0	.0	(?)	(?)	.2y	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Circenella sp	3514 Cl.	.3	.7	1.0	.....	.....	.....	(?)	.5	.8	(?)	.2	.7	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Mucor sp	3513	.3	.7	1.0	(*)	(*)	(*)	(?)	.2	1.0	.....	.....	.....	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Do	3541-6	.4	1.0	1.0	.....	.....	.....	.1—	.6	1.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Do	3560	.3	.7	1.0	.1y	.4	1.0	.1y	.7	1.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Do	3514 D4	.7	1.0	1.0	.....	.....	.....	.1+	1.0	1.0	.1+	1.0	1.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Rhizopus nigricans	.....	.7	1.0	1.0	(*)	(*)	(*)	(?)	.0	.0	(?)	.0	.0	.4	.9	1.0	.2—	.7	1.0	(?)	.0	.0	(?)	.0	.0
Syncephalastrum sp	.....	.6	.8	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.2	.5	.9	(?)	.5	.9	(?)	.0	.0	(?)	.0	.0
Fusarium sp	.....	.3	.7	.9	.....	.....	.....	.....	.....	.....	.....	.....	.....	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Oidium lactis	.....	.3	.5	.7	.2	.4	.7	.1	.2	.5	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0
Penicillium atramentosum	38	.2	.6	1.0	.2	.3	.8	.1—	.3	.7	(?)	.0+	.8	(?)	.4+	.7	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium brevicaule	2	.3	.6	1.0	.2	.4	.8	(?)	.2	.5	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....

[1] 1.0, a typical spore-bearing colony; 0.1, discernible germination of conidia; tenths, 0.1 to 1.0, relative amount of growth; ?, doubtful; 0, no growth; y, growth of a single spore; —, growth of a few widely scattered spores; +, growth of many spores; *, inharmonious results at times, but usually as given in the table.

TABLE I. —*Comparative effect of heating mold spores in milk to temperatures of from 120° to 150° F. (48.9° to 65.6 C.) for 30 minutes*—Continued

Name of mold.	Serial No.	Growth of spores when not heated (control).			Growth of spores when heated to temperature indicated and held for 30 minutes.																				
					120° F. (48.9° C.).			125° F. (51.7° C.).			130° F. (54.5° C.).			135° F. (57.2° C.).			140° F. (60.0° C.).			145° F. (62.8° C.).			150° F. (65.6° C.).		
		2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.
Penicillium camemberti	5	0.2	0.3	0.5	0.1	0.3	0.6	(?)	0.2y	0.3	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	.....	.....	.....
Penicillium camemberti, var. *rogri*	6	.2	.4	.7	.2	.5	.8	(?)	.2	.6	(?)	0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium chrysogenum	26	.3	.7	1.0	.3	.7	1.0	0.1—	.4	.8	(?)	(?)	.2—	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium citrinum	15	.2	.5	.8	(?)	.0	.0	(?)	.2—	.4	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium commune	23	.2	.4	.8	.....	.....	.....	.1	.4	.6	(?)	.2—	.3	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Penicillium cyclopium	2543a	.2	.5	.8	(?)	.2y	.4	(2)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Penicillium divaricatum	34	.3	.4	.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0.15	.3+	.4	(?)	.2—	.3	(?)	.0	.0
Penicillium expansum	14	.2	.5	.7	(?)	.3	.8	(?)	.2	.6	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium (*Citromyces*)	3523.4	.3	.5	.6	.....	.....	.....	.1	.4	.5	(?)	.3	.5	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium granulatum	9	.2	.6	.8	.....	.....	.....	.1	.3	.6	(?)	.1y	.2	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium lutcum	11	.2	.4	.7	.....	.....	.....	.1—	.3	.4	(?)	.3—	.4—	(?)	(?)	.3	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium notatum	102	.2	.5	1.0	.1	.4	.9	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium pinophilum	1	.2	.5	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Penicillium purpurogenum	17	.3	.5	.8	.....	.....	.....	(?)	.2	.5	(?)	.1y	.2	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium roqueforti	18	.2	.5	.8	.....	.....	.....	.1	.4	.8	(?)	.3—	.6	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium rugulosum	46	.2	.3	.7	.....	.....	.....	.1	.3	.6	(?)	.2—	.2	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Penicillium solitum	2526	.2	.5	.7	.2	.4	.8	(?)	.3	.4	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Penicillium spinulosum	45	.2	.4	.5	.....	.....	.....	.1—	.3	.4	(?)	.2y	.3	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium variabile	3551	.2	.5	.8	.2	.4	.9	(?)	.2—	.4	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Penicillium viridicatum	2552	.1	.2	.4	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Do	2643	.2	.4	.7	.1	.4	.8	(2)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Do	3028	.3	.6	.9	(?)	.3	.8	(?)	.1y	.2	(?)	.1y	.3	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Do	3514 A2	.2	.4	.8	.2	.6	.9	.1	.5	1.0	.....	.....	.....	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....
Penicillium asperulum	2683	.3	.5	.8	(?)	(?)	.3+	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Do	28	.2	.3	.5	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Do	83	.3	.6	.9	.1	.3	.3	(?)	.2y	.5	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Do	123	.4	.8	1.0	.....	.....	.....	.3	1.0	1.0	0.1—	.5	1.0	(?)	.0	.6	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Do	2670	.2	.5	.9	.....	.....	.....	.....	.....	.....	.1	.4	.8	(?)	.1—	.6	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Do	3525.62	.2	.5	.8	.....	.....	.....	.1+	.3	.6	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	(?)	.0	.0	.....	.....	.....
Do	3553	.4	.7	1.0	.....	.....	.....	.15+	.6	.9	(?)	.2—	.5	(?)	.3—	.6	(?)	.2	.8	(?)	.0	.0	.....	.....	.....
Do	3555.18	.2	.4	.7	.1+	.4	.8	.1	.5	.9	(?)	.0	.0	(?)	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Do	3555.19	.....	.....	.....	.....	.....	.....	.1	.9	1.0	(?)	.3	1.0	(?)	(?)	.3	(?)	.0	.0	.....	.....	.....	.....	.....	.....

A study of Table I shows that very few mold spores survive exposure to 140° F. (60° C.) in milk for 30 minutes and that at 145° F. (62.8° C.) still fewer are found. With reference to significant organisms, among the mucors the *Mucor racemosus* group (3513, 3523.6, 3560) and *Rhizopus nigricans*, which are found more frequently than all others of this group combined, were destroyed at 130° F. (54.5° C.). The common green species of Penicillium are mostly dead at 130° F. (54.5° C.); a few stand 135° F. (57.2° C.), but two, one of them an undescribed soil organism, survived 140° F. (60° C.) for 30 minutes. Among species of Aspergillus, however, the strains of *A. flavus*, *A. fumigatus*, and *A. repens* all survived 145° F. (62.8° C.) for 30 minutes; *A. repens* and *A. fumigatus* both survived 150° F. (65.6° C.). These three species are always found in forage and feeding stuffs; hence, milk is more or less subject to contamination with them. *A. repens* grows very poorly in milk, however, and the examination of a great many cultures of milk and its products has shown that the actual development of *A. flavus* and *A. fumigatus* is comparatively rare. Although these organisms grow at blood heat and have demonstrated their pathogenicity even to human beings at rare intervals as causes of disease in the lungs, there is no report of their growth in the alimentary canal.

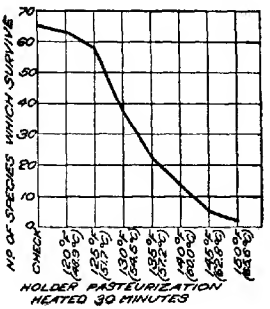

FIG. 1.—Curve of the number of species of molds surviving pasteurization of milk for 30 minutes at a series of temperatures.

The destruction of mold spores by the holder process of pasteurization is shown more clearly in figure 1, where the results have been plotted.

Pasteurization of milk at 145° F. (62.8° C.) may therefore be regarded as destroying mold spores completely enough to render them a negligible factor in the further changes found in the milk.

EXPERIMENTS WITH THE FLASH PROCESS OF PASTEURIZATION

In working with continuous pasteurizers, temperatures of 165° to 175° F. (73.9° to 79.5° C.) are reached by heating within a period of approximately 30 seconds and maintained about 30 seconds. This is followed by quick cooling. Lower temperatures have not been deemed satisfactory. A series of experiments was therefore planned to subject the freshly inoculated spores of species of Penicillium, Aspergillus, and of the mucors to these temperatures and to determine their relative ability to survive such heating. For this purpose glass tubing about 3 mm. in diameter was drawn into capillary form so that each tube had 3 or 4 inches of the original tub-

ing with 2 to 4 inches of capillary tube approximately 0.5 mm. in diameter. The open end of each tube was plugged with cotton. The tubes were packed into a copper case and dry-sterilized. For each experiment a few drops of sterile milk were transferred to the conidial surface of a colony and the conidia stirred into the milk. A column of milk 15 to 30 mm. long, bearing numerous conidia, was then drawn into the capillary tube and the end sealed in the flame. Experiments had shown that alcohol boiling at 172.4° F. (78° C.) when so treated would boil in 20 to 30 seconds when the tubes were thrust into water at 174.4° F. (79.1° C.). This showed that milk containing mold spores could be heated in from 20 to 30 seconds in capillary tubes to any given temperature when immersed in water 2 degrees Fahrenheit above the desired pasteurizing temperature. In our experiments, therefore, it was possible to duplicate flash pasteurization on a laboratory scale; for example, to pasteurize at 165° F. (73.9° C.) the capillary tubes containing milk and mold spores were held in water at 167° F. (75° C.) for 1 minute. During this period about 30 seconds were required to heat the milk and it was held at the pasteurizing temperature the other half minute. This is approximately the heating period of milk in commercial flash pasteurization. After heating for the required time, the tubes were cooled by thrusting them into cold water. The tip of the capillary was then broken off and the contents streaked upon slanted Czapek's solution agar. The slants were incubated, observed occasionally, and the results of the various experiments were tabulated separately and then brought together in Table II.

TABLE II.—*Comparative effect of heating mold spores in milk to temperatures of from 145° to 175° F. (62.8° to 79.5° C.) for 30 seconds* [1]

Name of mold.	Serial No.	Growth of spores.													
		Not heated (control).		Heated to 145° F. (62.8° C.).		Not heated (check).		Heated to 155° F. (68.3° C.).		Not heated (check).		Heated to 165° F. (73.9° C.).		Heated to 175° F. (79.5° C.).	
		6 days.	10 days.	6 days.	10 days.	3 days.	6 days.	3 days.	6 days.	4 days.	6 days.	4 days.	6 days.	4 days.	6 days.
Aspergillus candidus	106	0.4	0.7	0.8	1.0	0.3	0.7	0.0	0.0	.....					
Aspergillus flavus series	108	.9	1.0	.8	1.0	.4	.8	.0	.0	0.6	1.0	0.0	0.0	0.0	0.0
Do	3538, 108	.9	1.0	.6	1.0	.4	.8	.0	.0	.....		.....		.....	
Do	R4136	.8	1.0	.6	1.0	.5	.8	.0	.0	.....		.....		.....	
Do	Sc171	.7	.9	.0	.0	.5	.9	.0	.0	.....		.....		.....	
Aspergillus fumigatus	118	.9	1.0	.9	1.0	.3	.8	.0	.0	.6	1.0	.0	.0	.0	.0
Aspergillus glaucus?	2705	.8	1.0	.0	.0	.....	.....	.0	.0	.4	.8	.5?	1.0?	.0	.0
Do	3517	.....	.....	.....	.....	.3	.7	.0	.0	.....		.....		.....	
Do	3555-21	.8	.9	.0	.0	.3	.6	.0	.0	.....		.....		.....	
Aspergillus nidulans	110	.3	1.0	.3	1.0	.3	.8	.0	.0	.5	.9	.0	.0	.0	.0
Aspergillus niger	111	.9	1.0	.0	.0	.3	.7	.0	.0	.6	1.0	.0	.0	.0	.0
Aspergillus niger, var. *altipes*	3534-a	.9	1.0	.6	1.0	.5	.8	?	.4?	.....		.....		.....	
Aspergillus cinnamomeus	3534-b	.8	1.0	.6	1.0	.5	1.0	.0	.0	.5	1.0	.0	.0	.0	.0
Aspergillus fuscus	3534-c	.8	1.0	.7	.9	.....	.....	.0	.0	.7	1.0	.0	.0	.0	.0
Aspergillus ochraceus	112	.9	1.0	.0	.0	.9	1.0	.0	.0	.....		.....		.....	
Aspergillus oryzae	113	.8	1.0	.0	.0	.9	1.0	.0	.0	.5	.8	.0	.0	.0	.0

[1] 1.0, a typical colony; decimals, proportionate growth; 0.0, no growth; ?, inharmonious results.

TABLE II.—*Comparative effect of heating mold spores in milk to temperatures of from 145° to 175° F. (62.8° to 79.5° C.) for 30 seconds*—Continued

Name of mold.	Serial No.	Growth of spores.													
		Not heated (control).		Heated to 125° F. (62.8° C.).		Not heated (check).		Heated to 155° F. (68.3° C.).		Not heated (check).		Heated to 165° F. (73.9° C.).		Heated to 175° F. (79.5° C.).	
		6 days.	10 days.	6 days.	10 days.	3 days.	6 days.	3 days.	6 days.	4 days.	6 days.	4 days.	6 days.	4 days.	6 days.
Aspergillus repens						.8	1.0	0.8	1.0						
Aspergillus wentii	116	.8	1.0	.0	.0	.5	1.0	?	.5?						
Aspergillus sp.	Ra42	.7	1.0	.0	1.0	.8	1.0	.0	.0	.5	.8	.0	.0	.0	.0
Do	3522.30	.9	1.0	.0	.0	.9	1.0	.0	.0						
Do	3522.36														
Do	3336	.9	1.0	.0	.0	.5	1.0	.0	.0						
Aspergillus parasiticus	3509	.9	1.0	.6	1.0	.5	1.0	?	.6?	.7	1.0	.0	.0	.0	.0
Do	3505	.9	1.0	.5	1.0	.7	.9	.0	.0						
Circinella umbellata	3514.C1	.8	1.0	.8	1.0	.5	1.0	.0	.0	.8	1.0	.0	.0	.0	.0
Mucor racemosus (group)	3513	.8	.8	.8	1.0	.9	1.0	.0	.0	.7	1.0	.0	.0	.0	.0
Do	3523.6					.9	1.0	.0	.0						
Do	3560	1.0	1.0	1.0	1.0			.0	.0	.9	1.0	.0	.0	.0	.0
Rhizopus nigricans	3Rn.	.8	1.0	.0	1.0	.6	1.0	.0	.0	.7	1.0	.0	.0	.0	.0
Synephalastrum sp.	Syn.	.9	1.0	.5	1.0			.0	.0						
Fusarium sp.		.8	1.0	.9	1.0	.5	1.0	?	.7						
Penicillium atramentosum	38	.8	1.0	9.0	1.0					.5	1.0	.0	.0	.0	.0
Penicillium biforme	39	.9	1.0	1.0	.0	.3	.6	.0	.0	.5	1.0	.0	.0	.0	.0
Penicillium brevicaule	2	.3	bact.	.0	.0	.3	.6	.0	.0	.5	.6	.0	.0	.0	.0
Penicillium camemberti	5	.9	1.0	.0	.0	.9	1.0	.0	.0	.3	.7	.0	.0	.0	.0
Penicillium camemberti, var. *rogeri*	6	.8	1.0	.0	.0	.4	.6	.0	.0	.4	.9	.0	.0	.0	.0
Penicillium chrysogenum	26	.8	1.0	.0	.0	.4	.6	.0	.0	.0	.0	.0	.0	.0	.0
Penicillium citrinum	15	.7	1.0	.0	.7			.0	.0	.9	1.0	.0	.0	.0	.0
Penicillium commune	23	.8	1.0	.8	.8	.9	1.0	.0	.0	.7	1.0	.6?	1.0	.0	.0
Penicillium cyclopium	2543-a	.8	1.0	.4	.8	.5	.6	.0	.0	.5	1.0	.0	.0	.0	.0
Penicillium digitatum	16	.6	1.0	.0	.0										
Penicillium divaricatum	34	.9	1.0	.9	1.0	.4	.6	.0	.0	.8	.9	.0	.0	.0	.0
Penicillium duclauxi	20	.8	1.0	.4	.5	.5	.9	.0	.0						
Penicillium expansum	14					.5	.9	.0	.0	.6	.8	.0	.0	.2?	.5?
Penicillium (Citromyces) sp.	3523.4	.9	1.0	.9	1.0	.5	.9	.0	.0	.4	.8	.0	.0	.0	.0
Penicillium granulatum	9	.9	1.0	.0	.0	.5	.9	.0	.0	.4	1.0	.0	1.0	.0	.0
Penicillium italicum	10	.4	.9	.5	1.0	.3	.6	.0	.0						
Penicillium luteum	11	.8	.9	.4	1.0	.4	.8	Very slow.		.5	.9	?	?	.0	.0
Penicillium notatum	102	.6	.7	.1	.5	.3	.8	.0	.0	.3	.7	.0		.0	.0
Penicillium oxalicum	103	.8	.9	?	.7	.8	.8	.4	.8	.9	.0	.0		.0	.0
Penicillium pinophilum	1	.4	.8	.4	.8	.4	.9	.0	.0	.3	.7	.0	.0	.0	.0
Penicillium puberulum?	2651	.8	1.0	.0	.0	.1	.9	.0	.0						
Penicillium purpurogenum	17	.9	1.0	.0	.0	.3	.7	.0	.0	.6	.9	.0	.0	.0	.0
Penicillium purpurogenum, var. *rubri sclerotium*	2670					.3	.6	.0	.0	.5	1.0	.3	.5	.0	.6?
Penicillium roqueforti	18					.1	.8	.0	.0	.5	.9	.0	.0	.0	.0
Penicillium rugulosum	46	.4	.8	.4	.8					.3	.4	.0	.0	.0	.0
Penicillium solitum	2546	.8	1.0	.0	.0	.1	.6	.0	.0	.8	1.0	.0	.0	.0	.0
Penicillium solitum?	66	.9	1.0	.0	1.0	.5	.9	.0	.0	.5	1.0	.0	.0	.0	.0
Penicillium spindulosum	45	.8	1.0	?	.5					?	.8	.0	.0	.0	.0
Penicillium stoloniferum	27	.9	1.0	.4?	1.0	.4	.9	.0	.0	.1	.8	.0	.0	.0	.0
Penicillium variabile	3551	.9	1.0	.0	.0	.3	1.0	?	.7	.8	1.0	.0	.0	.0	.0
Penicillium viridicatum	2552	.9	1.0	?	.6	.3	.0	.0	.0						
Penicillium viridicatum, var. ?	2623	.9	1.0	.7	.8	.5	.7	.0	.0	.5	.7	.0	.0	.0	?
Do	3028	.7	1.0	.0	.0	.3	.8	.0	.0	.7	1.0	.8?	.9?	.0	.0
Do	3514-a	.8	1.0	.4?	.6?	.4	.9	.0	.0	.5	1.0	.0	.0	.0	.0
Penicillium (Citromyces) sp.	28			.6	.8	.3	.6	.0	.0	.4	.9	.0	.0	.0	.0
Do	63	.9	1.0	.0	.0	.4	.7	.0	.0	.8	1.0	.0	.0	.0	.0
Penicillium sp.	3525.61	.8	1.0	?	.4	.4	.8	.0	.0						
Do	3553					.5	.9	.0	.0	.0	.9	.0	.0	.0	.0
Do	3555.18									.4	.7	.0	.0	.0	.0
Do	3555.19	.7	.9	.4	.7	.6	.9	.0	.0						

From Table II it is seen that very few of the forms are killed in 30 seconds at 145° F. (62.8° C.); nearly all, however, are destroyed at 155° F. (68.3° C.). None of the colonies found at 165° F. (73.9° C.) and 175° F. (79.5° C.) were produced in both tubes. The chance of error is not fully eliminated in these cases. The consistent character of the whole table and the innocuous character of the few organisms in which occasional colonies occurred after heating show that temperatures of 165° to 175° F. (73.9° to 79.5° C.) for 30 seconds do practically destroy the spores of these molds as they may be found in milk, although a few conidia in some species may occasionally survive.

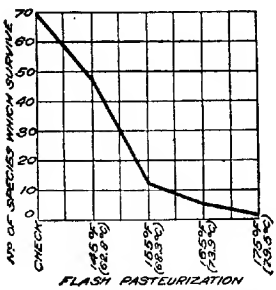

FIG. 2.—Curve of the number of species of molds surviving flash pasteurization at a series of temperatures.

Figure 2 shows graphically the effect of the flash process of pasteurization on mold spores.

DESTRUCTION OF MOLD SPORES BY DRY HEAT

The third series of experiments was planned to find the relative ability of the spores of approximately the same organisms to endure heating in dry air for the same period as used for heating in milk. After some experimentation the following method was used: Strips of heavy filter paper were cut wide enough so that only the edges would come into contact with the glass when dropped into test tubes. A drop of sterile water carrying a suspension of the spores under experiment was deposited in the middle of the paper strip and allowed to evaporate overnight. The tubes were then immersed in liquid heated to the desired temperature and held 30 minutes after check tubes carrying thermometers indicated that the air in the tubes had reached the same degree. The tubes were then removed and cooled. Melted agar was allowed to run into each tube to form a slant and the cultures were set away at room temperature. Observations of growth were made as in the previous experiments and the results tabulated in the same manner in Table III.

TABLE III.—*Comparative ability of mold spores to survive heating in dry air for 30 minutes at temperatures of 180° to 250° F. (82.2° to 121.1° C.)*[1]

Name of mold.	Serial No.	Growth of spores when not heated (control) and after having been heated to the temperature indicated for 30 minutes.																					
		Not heated (control) 5 days.	Heated to 180° F. (82.2° C.) 5 days.	Not heated (control) 7 days.	Heated to 190° F. (87.8° C.).		Not heated (control).		Heated to 200° F. (93.3° C.).		Not heated (control).		Heated to 210° F. (98.9° C.).		Heated to 220° F. (104.5° C.).		Not heated (control).		Heated to 230° F. (110.0° C.).		Not heated (control).		Heated to 250° F. (121.1° C.).	
					3 days.	7 days.	4 days.	8 days.	4 days.	8 days.	4 days.	7 days.	4 days.	7 days.	4 days.	7 days.	3 days.	6 days.	3 days.	6 days.	4 days.	8 days.	4 days.	8 days.
Aspergillus candidus	106	p. 5	0.3	p. 4	....	0.0	....	....	....	....	0.1	....	....	....	....	....	?	0.6	?	?0.0	?	0.4	0.0	0.0
Aspergillus flavus, var	108	.7	.7	....	....	....	0.3	....	....	0.1	0.7	....	0.2	0.9	....	....	0.2	.9	0.3	....	0.5	.8	.0	.0
Do	3538. 108	.5	.5	....	....	....	....	....	....	....	.5	....	?	.5	....	....	.3	.9	.5	1.0	.5	.8	?	.6?
Do	R3136	.8	.6	.8	....	.3	.4	....	....	.0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Do	Sc171	.5	.4	....	....	....	.4	....	0.3	.4	.4	....	....	....	....	....	.2	.9	.0	.0	....	....	....	....
Aspergillus fumigatus	118	.8	.6	....	....	....	.3	....	?	.6	.5	....	.1	.8	0.3	....	.2	.9	.3	1.0	.5	.9	.0	.0
Aspergillus glaucus	2705	.6	.4	....	....	....	.2	....	?	.2	.8	....	.0	.0	....	....	.2	.8	.0	.0	.3	.7	.0	.0
Aspergillus glaucus ?	3512	.8	.4	.8	....	.0	.4	....	.0	.0	....	....	....	....	....	....	?	.9	.2	1.0	.4	.9	.0	.0
Aspergillus versicolor ?	3555. 21	.5	.5	.8	....	.0	.3	....	.0	.0	....	....	....	....	....	....	.2	.9	?	?	.3	.6	.0	.0
Aspergillus nidulans	110	.5	.5	....	....	....	.2	....	?	.4	.6	....	.0	.0	....	....	.3	.9	?	?	.4	.8	.0	.0
Aspergillus niger	111	.7	.5	....	....	....	.4	....	?	.3	.8	....	.0	.0	....	....	.3	.9	.5	.8	....	....	....	....
Aspergillus niger, var. *altipes*	3534a	.8	.6	....	....	....	.3	....	?	.5	....	....	....	....	....	....	.2	1.0	.6	1.0	.4	.8	.0	.0
Aspergillus cinnamomeus	3534b	.7	.3	....	....	....	.3	....	?	.4	.8	....	?	....	.3	....	.2	.9	.6	.8	.4	.7	.0	.0
Aspergillus fuscus	3534c	.8	....	....	....	....	.5	....	?	.4	.7	....	.0	.0	....	....	.2	.9	.5	1.0	.4	.7	.0	.0
Aspergillus ochraceus	112	.7	.4	.8	....	.0	.4	....	.0	.0	....	....	....	....	....	....	.3	.9	?	?	....	....	....	....
Aspergillus oryzae	113	.8	.5	....	....	....	.3	....	?	.4	.8	....	.0	.0	.2	....	.3	.7	.0	.0	....	....	....	....
Aspergillus repens	....	.4	.2	....	....	....	....	....	....	....	.4	....	.0	.0	....	....	....	....	....	....	.2	.4	.0	.0
Aspergillus wentii	116	.7	.0	.7	....	.0	.5	....	.0	.0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Aspergillus sp	Ra42	.6	.3	.5	....	.3	.2	....	.0	.0	....	....	....	....	....	....	.2	.8	.0	.0	....	....	....	....
Do	3522. 30	.8	.4	....	....	....	.2	....	.4	.5	.7	....	.0	.0	....	....	.2	.8	?	.3	.3	.6	.0	.0
Do	3522. 36	.5	.2	.5	....	.0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Do	3556	.9	.6	....	....	....	.4	....	?	.6	.8	....	.0	.0	....	....	.3	.9	.3	.5	.4	.8	.0	.0
Aspergillus parasiticus	3509	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	.3	.9	.3	1.0	.4	.7	.0	.0
Aspergillus sp	3565	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	.3	.9	.3	1.0	.4	.7	.0	.0
Circinella umbellata	3514 C1	....	....	....	....	....	.3	....	?	.4	.6	....	?	.6	.3	0.7	.5	.9	.5	.8	.3	.7	?	.4
Mucor racemosus ?	3513	....	....	....	....	....	....	....	....	....	.4	....	.4	.8	....	.5	.4	.9	.7	1.0	.4	.7	?	.8
Do	3523. 6	.6	.5	....	....	....	.3	....	.3	2.0	.6	....	?	.7	....	....	.4	.9	.9	.8	.4	.8	.0	.0
Do	3560	.7	....	●	....	....	.3	....	.4	.4	.8	0.9	?	.9	.8	.8	.5	1.0	.9	.9	.6	1.0	?	1.0
Rhizopus nigricans	....	.6	.6	....	....	....	.3	0.5	.3	.5	.3	.9	.3	.9	.3	.7	.3	.8	.6	.9	.3	.6	.0	.0
Syncephalastrum sp	....	.5	.5	....	....	....	.2	....	?	.4	?	.9	?	.6	?	?	.2	.9	?	.5	....	....	....	....
Fusarium sp	....	.7	.5	....	....	....	.5	.8	.4	.8	....	.7	.5	.8	....	....	.2	.8	?	.6	.4	.6	.0	.0

[1] 1.0, a typical colony: decimals, proportionate growth, 0.0, no growth; ? inharmonious results: y, growth of a single spore,

TABLE III.—*Comparative ability of mold spores to survive heating in dry air for 30 minutes at temperatures of 180° to 250° F. (82.2° to 121.1° C.)*—Contd.

Name of mold.	Serial No.	Growth of spores when not heated (control) and after having been heated to the temperature indicated for 30 minutes.																					
		Not heated (control) 5 days.	Heated to 180° F. (82.2° C.) 5 days.	Not heated (control) 7 days.	Heated to 190° F. (87.8° C.).		Not heated (control).		Heated to 200° F. (93.3° C.).		Not heated (control).		Heated to 210° F. (98.9° C.).		Heated to 220° F. (104.5° C.).		Not heated (control).		Heated to 230° F. (110.0° C.).		Not heated (control).		Heated to 250° F. (121.1° C.).	
					3 days.	7 days.	4 days.	8 days.	4 days.	8 days.	4 days.	7 days.	4 days.	7 days.	4 days.	7 days.	3 days.	6 days.	3 days.	6 days.	4 days.	8 days.	4 days.	8 days.
Penicillium atramentosum	38	0.7	0.5	0.9	0.3	0.6	0.4	.....	0.0	0.0	.....	.....	.....	.....	.....	.....	?	0.8	0.0	0.0	.....	.....	.....	.....
Penicillium brevi-caule	37	.....	.....	.....	.....	.....	.6	.....	.0	.0	0.7	.....	0.0	0.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium camemberti	2	.3	.0	.7	.0	.0	.4	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium camemberti var. *roqueti*	5	.7	.3	.8	.0	.0	.3	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium chrysogenum	6	.0	.3	.8	?	?	.3	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium citrinum	20	.3	.8	.....	.....	.....	.7	.....	?	.7?	.8	.....	.4	.6	.....	.....	.3	.9	.4	1.0	.5	.9	.0	.0
Penicillium commune	13	.8	.0	.9	.0	.0	.5	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium cyclopium	21	.8	.4	.7	.0	.0	.6	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium digitatum	254.6	.8	.....	.....	.....	.....	?	.....	?	.2	.8	.....	.0	.0	.....	.....	.2	1.0	?	?	.4	.9	.0	.0
Penicillium divaricatum	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium avellanii	34	.7	.6	.....	.....	.....	.3	.....	?	.6	.8	.....	?	.3	.....	.....	.2	1.0	.7	1.0	.3	.7	.0	.0
Penicillium expansum	20	.6	.5	.....	.....	.....	.3	.....	?	.1	.5	.....	.4	.7	.....	.....	.3	.9	?	?	.....	.....	.....	.....
Penicillium gilvum (*Citromyces*)	14	.....	.....	.7	?	.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium granulatum	3323.4Y	.9	.6	.9	?	.2	.5	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium italicum	9	.7	.....	.9	.0	.0	.5	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium luteum	11	.8	.4	.3	?	.3	.3	.....	?	.4	.....	.....	.....	.....	.....	.....	.3	.9	.0	.0	.....	.....	.....	.....
Penicillium notatum	102	.8	.6	.7	?	.4	.6	.....	?	1.0	.8	.....	.4	.8	.0	.0	.2	.9	?	?	.....	.....	.....	.....
Penicillium oxalicum	103	.8	.7	.....	.....	.....	.7	.....	?	.4	.8	.....	.4?	.8	.....	.....	.....	.....	.....	.....	.4	.8	.0	.0
Penicillium pinophilum	1	.5	.4	.....	.....	.....	.4	.....	?	.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium puberulum	2683	.....	.....	.8	?	.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium purpurogenum	17	.7	.3	.7	.1	.4	.3	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium purpurogenum, var. *rubri-sclerotium*	2670	.....	.....	.6	.4	.9	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium roqueforti	18	.7	.4	.6	.0	.0	.4	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium rugulosum	46	.5	.3	.8	?	.4	.3	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium solitum	2546	.7	.4	.8	.0	.0	.5	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium solitum ?	66	.....	.....	.....	.....	.....	.6	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium spinulosum	44	.5	.3	.....	.....	.....	.3	.....	?	.3?	.7	.....	.1	.5	.....	.....	.3	.9	.6	1.0	.8	.8	.0	.0
Penicillium stoloniferum	27	.....	.....	.8	.0	.0	.7	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Penicillium variabile	3551	.7	.5	.....	.....	.....	.5	.....	?	.3	.7	.....	.0	.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

| | | |

A study of Table III shows that mold spores possess much greater ability to withstand dry heat than heating in milk. Very few forms were destroyed at 180° F. (82.2° C.), but they include *Penicillium brevicaule*, which has a thick-walled spore and in laboratory cultures has remained viable at least 7 years. Only a few species of Penicillium survived heating to 200° F. (93.3° C.) for 30 minutes. All these are forms which grew at 98.6° F. (37° C.), and some of them are widely distributed.

Aside from *A. wentii*, all the species of Aspergillus survived heating at 200° F. (93.3° C.). Several of them survived at 230° F. (110° C.), but after 250° F. (121.1° C.) for 30 minutes no species showed growth after 6 days' incubation. Three of six mucors, however, survived the heating to 250° F. (121.1° C.) for 30 minutes. These species were killed quickly by both forms of heating in milk. The results of these experiments are plotted in figure 3.

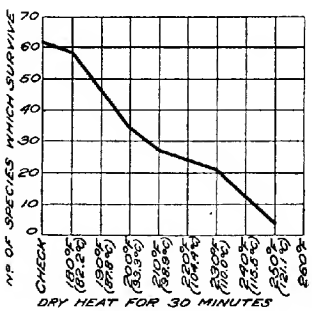

FIG. 3.—Curve of the number of species of molds surviving dry heat for 30 minutes at a series of temperatures.

The destruction of mold spores by dry heat has no relation to the subject of pasteurization of milk, but it is of scientific interest.

DISCUSSION OF RESULTS

These results with mold spores agree in general with bacteriological studies of pasteurization. Very few of these organisms found in milk survive after 30 minutes' heating to 145° F. (62.8° C.). Certain molds, notably *Aspergillus fumigatus* and *A. flavus*, do survive, but they occur only occasionally in milk. *Oidium lactis* and the mucors, which are probably more important as milk-borne organisms than all the rest, are destroyed at the low temperatures used in the holder process of pasteurization. In the flash process very few mold spores survived at 165° F. (73.9° C.). Occasionally some spores seem to have escaped destruction at 175° F. (79.5° C.), but the organisms surviving in these cases were of minor importance in the decomposition of dairy products. In confirmation of these results the writers have had access to unpublished data of Mr. R. O. Webster, of the Bureau of Chemistry, giving cultural analysis of butter made from flash-pasteurized cream on a commercial basis. Cultures from this butter showed no mold spores, while cultures made at the same time from country butter showed 20,000 to 60,000 per gram.

Mold spores in milk seem, therefore, to be destroyed completely or reduced to negligible numbers by both of the standard pasteurization processes.

Careful study of the cultures showed that the first effect of heating was to delay germination. This is indicated in the tables by the reports of successive examinations of the same culture. In Table I three reports are given; later only two reports. The third and fourth observations, however, were usually made. At times heating to a degree just under the death point delayed germination almost the full length of the usual growing period of the species. The number of possible sources of error was so great that the results of observations have been tabulated and compared. When essential harmony of results was not obtained, the work was repeated. In a few cases the continued lack of consistent results for particular organisms is indicated by the interrogation point in the tables. Even with these precautions the data obtained can be said to apply only to the strains used. This is indicated by comparing the results given for the *Aspergillus flavus* group or for the four members of the *A. niger* group. These results do not prove that other strains of these groups would respond exactly as here tabulated. In fact, more extended studies (as yet unpublished) of these two groups indicate that organisms otherwise undistinguishable may differ greatly if we measure a single physiological reaction. Such quantitative differences may persist in continued cultures, but are hardly comparable to differences in the kind of reaction as a basis for separating species. Inside the race or strain, conidia transferred from the same culture respond very differently. There is frequently a survival of a few spores where a majority of the spores die. There may be, therefore, a difference of as much as 20° F. (11.1 C.) between the temperature at which an occasional culture is completely killed and that at which cultures of that species are uniformly killed. These results resemble those obtained in determining the thermal death point of bacteria.

The applicability of these results to the occurrence of mold spores in substances other than milk has not been tested. The variation in composition of the substratum together with the heating may at times introduce a considerable variation. In general, however, it is clear that mold spores are easily killed by heat when suspended in fluid. The tables have been studied in an attempt to correlate resistance with size of spore or thickness of spore wall. No such correlation has been found. There is, therefore, no suggestion as to the nature of the difference in these organisms which affects their resistance to heat.

SUMMARY

(1) The holder process of pasteurization, in which milk was heated to 145° F. (62.8° C.) and maintained at that temperature for 30 minutes, killed the conidia of every species investigated, except those of *Asper-*

gillus repens, *A. flavus*, and *A. fumigatus*. The molds which survive are found only occasionally in milk.

(2) The flash process of pasteurization, where milk was heated to 165° F. (73.9° C.) for a period of 30 seconds, destroyed the spores of all the molds tested with the exception of many spores of one form and occasional spores of three more forms. At 175° F. (79.5° C.) only occasional spores of two forms developed.

(3) When the heating process was performed in dry air for a period of 30 seconds at 200° F. (93.3° C.), 31 out of 42 forms of Penicillium and 7 out of 24 forms of Aspergillus were destroyed, but none of the cultures of the mucors. A temperature of 250° F. (121.1° C.) over a period of 30 minutes killed all the forms of *Penicillium* spp. tried, but left an occasional living spore in one species of Aspergillus and three out of six mucors.

EFFECT OF WATER IN THE RATION ON THE COMPOSITION OF MILK

By W. F. TURNER, R. H. SHAW, R. P. NORTON, and P. A. WRIGHT, *of the Dairy Division, Bureau of Animal Industry*

INTRODUCTION

Experiments conducted at Brownsville, Tex., by the Dairy Division of the Bureau of Animal Industry indicate that the feeding of prickly-pear (*Opuntia* spp.) lowers the percentage of fat in milk. In comparison with other feeds prickly-pear contains a large amount of water and mineral matter. It was thought by the writers that one or both of these constituents might be responsible for the reduction in fat percentage; consequently experiments were conducted at Beltsville, Md., to determine the influence of the water. Work with the mineral matter is now in progress.

The literature dealing with the effects of watery feeds or water in the ration upon the quantity and the quality of milk produced contains many conflicting statements. No doubt the difficulty of eliminating all factors except the watery character of the ration is largely responsible for the conflicting nature of these statements.

Gilchrist (1)[1] reports very little difference, if any, in quantity and quality between the milk produced by cows either on pasture only or on a daily ration of mangels in varying amounts up to 86 pounds per cow and that produced by the same cows on a ration of hay and grain.

Tangl and Zaitschek (12) state, as the result of extensive experiments to determine the influence of watery feeds on milk secretion, that there is no difference between the composition of the milk from cows fed on a watery ration and that from cows fed on a dry one. They state that it is not true that watery feeds cause the production of thinner milk than dry feeds.

Lauder and Fagan (10, p. 9) reached the following conclusions from experiments extending over a 3-year period, using 60 cows and feeding a large ration of turnips (*Brassica rapa*) to compare with a dry or concentrated ration:

> The feeding of a ration containing a large quantity of water does not increase the percentage of water in the milk or reduce the percentage of fat.
>
> The greater yield of milk was obtained from the cows on the concentrated ration. On the other hand, the milk from the cows on the turnip ration contained a higher percentage of fat, and a greater total weight of fat was secreted in the milk.

[1] Reference is made by number to "Literature cited," p. 177-178.

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Holtsmark (6) reports that there is no decrease in the fat content of the milk of cows on a liberal daily ration of concentrated feed and cut straw, with as much as 77 pounds of turnips per head, after this ration is substituted for one consisting of hay, straw, concentrates, and a small quantity of roots.

A writer in the Journal of the Board of Agriculture (3), London, England, concludes from a study of the work of various investigators that, although many feeds have a specific effect on the yield and quality of milk, it may be attributed to stimulating substances in the feeds rather than to water content. These substances have a physiological rather than a nutritive effect and are present in feeds in small quantities only.

As the result of a number of experiments conducted and a review of previous work of the same character, Jordan (8, p. 69) states that, "Contrary to a notion held by many, it is not possible to water a cow's milk through her drink or through the ingesting of watery feed."

The Journal of the Board of Agriculture, London (2), reports that a dairyman was convicted in the French courts for selling adulterated milk. The conviction was based upon the assumption that it is possible to water milk either by feeding cows on watery feeds, by causing them to drink water in large quantities, or by making them drink immediately before milking. To prove the fallacy of this assumption, the Board conducted experiments with a number of cows. After feeding them an excess of common salt (sodium chlorid), or limiting the water drunk after free access to it, or permitting them to drink only immediately before milking, it was found that no change is produced in the composition of the milk.

At Offerton Hall, Durham, England, a series of experiments was conducted to determine how the composition of milk is affected by feeding wet brewers' grains. The first of these experiments (7, p. 35) indicates that the feeding of these grains to cows whose milk is habitually low in butter fat is not to be recommended, especially during the earlier stages of the lactation period, when the grains tend slightly to reduce the yield of fat. The writer advises dairymen to use such grains sparingly. Later experiments (13, p. 19–20) indicate that the grains may be fed safely if the ration contains other feeds also, and that there is no appreciable lowering of the butter fat when the grains are fed in moderate quantities.

In a general article upon the effect of different feeds upon the quality of milk, McConnell (11) says:

> It is a matter of common knowledge that the lush grass of spring, an excess of mangolds, or too many brewers' grains will promote a great flow of milk, but that that milk will be poor, and farmers who do not do anything to modify such feeding will find their milk coming dangerously near the "standard."

Hansson (4), of the Stockholm Agricultural Experiment Station, in a review of the work of various investigators concerning the effect of different feeds upon the fat content of milk, concludes that there are on

this point distinct differences among different feeds, but that the effect of any feed depends upon the composition of the other components of the ration. He states that roots have a favorable effect upon milk secretion, but tend slightly to lower the fat content.

Koch (9) reports extensive feeding experiments at Rosenhof in which cows were fed beet roots (*Beta vulgaris*), and gives the following conclusions:

> An increase in fat units (total fat) with beet-root feed, an increase of the amount of milk combined with a decrease in the fat content. However, the increase in quantity exceeded the decrease in quality so much that the cows gave 6 per cent more total fat on the beet-root ration.[1]

PLAN OF INVESTIGATION

The experimental work to determine the effect of water in the ration upon the composition of milk was conducted at the Dairy Division farm, Beltsville, Md., and included parts of three different lactation periods. The four following methods for supplying rations of widely different water content were tried:

1. A full allowance of drinking water as compared with a limited supply, the ration otherwise being alike in both cases.
2. A heavy ration of turnips as compared with a dry-roughage one.
3. Wet beet pulp as compared with dry beet pulp.
4. Green crimson clover (*Trifolium incarnatum*) as compared with the cured hay.

As the change in the fat content of the milk noted during the prickly-pear experiments took place within a few days after the change in the character of the ration and continued throughout the 80-day period, it was decided that for this work two 10-day periods of feeding any one ration, with a 10-day transition period intervening, and equal periods of feeding the comparative ration, would give time enough for any change in the composition of the milk to take place. In each series of experiments the milk from each cow was weighed at each milking, and 10-day composite samples were taken for analysis. The data obtained from each series of experiments are given separately.

FULL VERSUS LIMITED ALLOWANCE OF WATER

In this series of experiments eight cows were used and all received the same general treatment. For the first two 10-day periods the animals were given water ad libitum twice daily. Then a definite quantity of water, not more than 75 per cent of the full allowance, and in some cases less than 65 per cent, was given for two 10-day periods following a 10-day transition period. The quantity of water given in the limited water ration was so reduced that, when watered once a day, all cows drank the quantity allowed. After a second 10-day transition period, a full allowance of water was again given for two 10-day periods. This completed the work

[1] Authors' translation.

with all but two cows, which were given a still more reduced allowance of water following the second full-allowance period. Table I gives the results for each cow.

TABLE I.—*Comparison of the effect of a full and a limited allowance of water on the composition of milk*

COW 100

Water allowance.	Total milk.	Total water.	Fat.		Specific gravity.	Solids not fat.	Moisture.	Ash.	Total protein.
	Pounds.	*Pounds.*	*Per cent.*	*Pounds.*		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Full	220.6	412.5	4.50	9.93	1.033	9.11	86.39	0.720	3.35
Do.	240.6	502.0	4.50	10.83	1.032	9.19	86.31	.710	3.35
Transition	205.3	340.0	4.60	9.44	1.033	9.27	86.13	.720	3.36
Limited	198.8	340.0	4.80	9.54	1.034	9.36	85.84	.710	3.52
Do.	199.3	340.0	4.70	9.37	1.033	9.18	86.12	.705	3.53
Transition	197.6	434.0	4.35	8.60	1.032	9.11	86.54	.700	3.41
Full	172.2	378.0	4.90	8.44	1.032	9.01	86.09	.745	3.68
Do.	167.0	358.0	4.70	7.85	1.033	9.18	86.12	.747	3.66
Transition	149.8	200.0	4.88	7.19	1.033	9.36	85.76	.755	3.80
Limited	135.8	205.0	4.90	5.65	1.032	9.30	85.80	.770	3.72
Do.	138.0	215.0	4.80	6.62	1.032	9.13	86.07	.750	3.61
Average:									
Full	200.1	412.5	4.65	9.26	.....	9.12	86.23	.730	3.51
Limited	168.0	273.0	4.80	7.79	.....	9.24	85.96	.734	3.59

COW 21

Full	191.8	500.5	6.00	11.51	1.036	9.98	84.02	.770	3.92
Do.	188.0	502.0	6.00	10.88	1.035	10.14	83.86	.770	3.94
Transition	179.0	320.0	6.25	11.19	1.036	10.18	83.57	.750	3.94
Limited	173.2	320.0	6.10	10.69	1.035	10.11	83.79	.740	3.87
Do.	179.0	320.0	6.03	10.79	1.035	10.21	83.76	.740	3.96
Transition	181.6	496.0	5.50	9.99	1.034	9.91	84.59	.730	3.82
Full	184.5	462.0	5.73	10.57	1.035	10.00	84.27	.720	3.90
Do.	180.4	473.0	6.00	10.82	1.035	9.81	84.19	.740	3.99
Transition	172.9	230.0	5.95	10.29	1.035	9.95	84.07	.755	3.91
Limited	157.8	255.0	6.00	9.47	1.034	10.10	83.90	.750	3.91
Do.	151.5	280.0	5.80	8.79	1.033	9.82	84.38	.730	3.76
Average:									
Full	186.2	484.0	5.93	10.94	.....	9.98	84.08	.750	3.94
Limited	165.9	294.0	5.98	9.94	.....	10.06	83.96	.740	3.88

COW 19

Full	220.5	520.0	5.30	11.69	1.036	9.83	84.87	.770	3.73
Do.	228.6	492.0	5.20	11.89	1.035	9.82	84.98	.745	3.86
Transition	213.0	300.0	5.18	11.03	1.036	10.09	84.73	.735	3.73
Limited	213.1	345.0	5.42	11.55	1.035	9.66	84.92	.750	3.77
Do.	202.5	305.0	5.33	10.79	1.035	9.98	84.69	.780	3.82
Transition	203.3	622.0	5.60	11.38	1.034	9.66	84.74	.765	3.83
Full	198.5	520.0	5.50	10.92	1.034	9.74	84.76	.775	3.88
Do.	193.2	520.0	5.25	10.14	1.034	9.71	85.04	.765	3.74
Average:									
Full	205.2	513.0	5.31	11.16	.....	9.77	84.91	.764	3.80
Limited	207.8	325.0	5.37	11.17	.....	9.82	84.80	.765	3.79

TABLE I.—*Comparison of the effect of a full and a limited allowance of water on the composition of milk*—Continued

COW 8

Water allowance.	Total milk.	Total water.	Fat.		Specific gravity.	Solids not fat.	Moisture.	Ash.	Total protein.
	Pounds.	*Pounds.*	*Per cent.*	*Pounds.*		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent*
Full	252. 2	573. 0	4. 55	11. 48	1. 033	9. 30	86. 15	0. 730	3. 17
Do	253. 0	553. 0	4. 15	10. 50	1. 033	9. 10	86. 75	. 723	3. 12
Transition	234. 9	346. 0	4. 20	10. 46	1. 034	9. 39	86. 41	. 707	3. 20
Limited	220. 7	350. 0	4. 30	9. 49	1. 034	9. 44	86. 26	. 731	3. 14
Do	208. 0	350. 0	4. 65	9. 67	1. 034	9. 35	86. 00	. 714	3. 11
Transition	217. 6	509. 0	4. 30	9. 37	1. 033	8. 98	86. 72	. 727	3. 11
Full	205. 7	500. 0	4. 30	8. 85	1. 034	9. 25	86. 45	. 724	3. 06
Do	209. 4	536. 0	4. 50	9. 42	1. 033	9. 18	86. 32	. 732	3. 17
Average:									
Full	230. 1	540. 0	4. 37	10. 06	.....	9. 21	86. 88	. 727	3. 13
Limited	214. 3	350. 0	4. 47	9. 58	.....	9. 39	86. 13	. 722	3. 12

COW 17

Full	194. 9	465. 0	5. 30	10. 33	1. 034	9. 73	84. 97	. 74	3. 70
Do	206. 8	437. 0	4. 95	10. 24	1. 035	9. 88	85. 17	. 72	3. 77
Transition	173. 5	267. 0	5. 28	9. 02	1. 035	9. 73	84. 99	. 715	3. 62
Limited	188. 2	310. 0	5. 15	9. 69	1. 034	9. 53	85. 32	. 720	3. 68
Do	174. 0	300. 0	5. 20	9. 05	1. 035	9. 92	84. 88	. 755	3. 68
Transition	184. 7	443. 0	5. 18	9. 57	1. 034	9. 44	85. 38	. 740	3. 81
Full	164. 9	499. 0	5. 20	8. 57	1. 033	9. 69	85. 11	. 770	3. 86
Do	156. 4	504. 0	5. 18	8. 10	1. 033	9. 70	85. 12	. 755	3. 76
Average:									
Full	180. 7	476. 0	5. 16	9. 31	.....	9. 75	85. 09	. 744	3. 77
Limited	181. 1	305. 0	5. 17	9. 37	.....	9. 72	85. 10	. 737	3. 68

COW 9

Full	199. 7	410. 0	4. 40	8. 79	1. 031	8. 83	86. 77	. 744	2. 78
Do	193. 7	432. 0	4. 15	8. 04	1. 030	8. 40	87. 45	. 709	2. 65
Transition	181. 0	386. 0	4. 20	7. 60	1. 031	8. 53	87. 27	. 711	2. 76
Limited	163. 5	300. 0	4. 05	6. 62	1. 032	8. 79	87. 16	. 724	2. 72
Do	142. 9	300. 0	4. 15	5. 93	1. 031	8. 64	87. 21	. 703	2. 54
Transition	165. 7	526. 0	4. 10	6. 79	1. 030	8. 27	87. 63	. 698	2. 69
Full	170. 4	556. 0	4. 15	7. 07	1. 031	8. 53	87. 32	. 712	2. 73
Do	164. 9	541. 0	4. 30	7. 09	1. 031	8. 65	87. 05	. 704	2. 84
Average:									
Full	182. 2	485. 0	4. 25	7. 75	.....	8. 60	87. 15	. 717	2. 75
Limited	153. 2	300. 0	4. 10	5. 77	.....	8. 71	87. 18	. 713	2. 63

COW 14

Full	269. 1	429. 0	5. 10	13. 72	1. 033	9. 17	85. 73	. 723	3. 04
Do	263. 9	470. 0	4. 80	12. 67	1. 032	9. 11	86. 09	. 723	3. 10
Transition	236. 5	338. 0	5. 40	12. 77	1. 033	9. 10	85. 50	. 754	3. 18
Limited	232. 9	305. 0	5. 00	11. 64	1. 033	8. 97	86. 03	. 756	3. 22
Do	222. 6	300. 0	5. 10	11. 36	1. 033	8. 91	85. 99	. 739	3. 25
Transition	235. 2	566. 0	4. 90	11. 52	1. 032	8. 84	86. 26	. 727	3. 16
Full	227. 6	494. 0	5. 05	11. 50	1. 032	9. 06	85. 89	. 737	3. 21
Do	211. 1	484. 0	4. 70	9. 92	1. 031	8. 99	86. 31	. 724	3. 06
Average:									
Full	242. 9	469. 0	4. 91	11. 95	.....	9. 09	86. 00	. 727	3. 10
Limited	227. 7	302. 0	5. 05	11. 50	.....	8. 94	86. 51	. 747	3. 23

TABLE I.—*Comparison of the effect of a full and a limited allowance of water on the composition of milk*—Continued

COW 2

Water allowance.	Total milk.	Total water.	Fat.		Specific gravity.	Solids not fat.	Moisture.	Ash.	Total protein.
	Pounds.	*Pounds.*	*Per cent.*	*Pounds.*		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent*
Full.....	238. 6	517. 0	5. 60	13. 37	1. 033	9. 55	84. 85	0. 724	3. 50
Do.....	236. 5	595. 0	4. 85	11. 47	1. 034	9. 68	85. 47	.737	3. 67
Transition....	201. 6	388. 0	5. 60	11. 29	1. 035	9. 92	84. 48	.775	3. 85
Limited.....	181. 9	350. 0	5. 50	10. 00	1. 035	9. 57	84. 93	.769	3. 82
Do.....	188. 5	350. 0	5. 55	10. 47	1. 035	9. 57	84. 88	.746	3. 77
Transition....	172. 7	589. 0	5. 30	9. 15	1. 032	9. 32	85. 38	.738	3. 48
Full.....	189. 9	630. 0	4. 95	9. 40	1. 034	9. 54	85. 51	.754	3. 56
Do.....	202. 9	639. 0	4. 70	9. 54	1. 033	9. 62	85. 68	.747	3. 89
Average:									
Full.....	217. 0	595. 0	5. 02	10. 94	.....	9. 60	85. 38	.740	3. 65
Limited..	185. 2	350. 0	5. 52	10. 23	.....	9. 57	84. 90	.757	3. 79

In studying the data obtained in these trials it will be noted that all the milk constituents except the fat show very little variation during the different periods, and that these differences are attributable more to the individual animals than to the character of the ration. Taking the average figures for the two classes of rations, it will be seen that the full water allowance ration tended to increase the quantity of milk produced and to cause a slight reduction of the fat content of the milk. A study of the data for individual cows by separate periods, however, will show that this average effect of the different rations is caused more by the order in which the rations are fed than by their character. Of the data obtained from the eight cows used in this test those from only one (No. 2) show indication of any effect of the ration upon the composition of the milk, and the data from the seven other cows are so negative that this variation is probably caused more by the individual than by the ration. Two of the cows, Nos. 17 and 19, show practically no variation in either quantity or quality of the milk produced; one other, No. 100, decreased gradually in the quantity of milk produced and increased gradually in quality, regardless of the ration; while the remaining four, Nos. 8, 9, 14, and 21, gave milk the fat content of which varied considerably from normal in different periods, even on the same ration. These variations were independent of the character of the ration—that is, the abnormal percentage of fat was in some cases found when the full allowance of water was given and in other cases when the quantity was reduced. A summing up of all the data obtained shows that the feeding of rations whose water content is varied by controlling the quantity of water drunk has no influence upon the composition of the milk produced.

TURNIPS VERSUS DRY-ROUGHAGE RATION

In this series of experiments four cows were used, the experimental period consisting of six test periods and two transition periods. Figure 1 shows the grouping of the cows and the character of the ration fed during each period.

As much as 90 pounds of turnips a day was fed to the cows on the wet-roughage ration, with the addition of 4 pounds of clover hay. The roughage ration of the dry-roughage group consisted entirely of clover hay. The grain ration was the same for both groups. In Table II

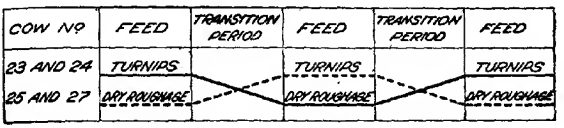

FIG. 1.—Grouping of cows and kind of ration fed cows 23, 24, 25, and 27

both the quantity of water drunk and the total water content of the turnips are given, turnips being considered as having 90 per cent of water, as shown by Henry and Morrison (5, p. 645).

TABLE II.—*Comparison of the effect of turnips and a dry-roughage ration on the composition of milk*

COW 23

Ration.	Total milk.	Water in ration.	Turnips.	Fat.		Specific gravity.	Solids not fat.	Moisture.	Ash.	Total protein.
	Lb.	*Lb.*	*Lb.*	*P. ct.*	*Lb.*		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Wet	234.7	94	774	4.10	9.62	1.032	8.64	87.26	0.750	3.18
Do	236.9	123	810	4.30	10.19	1.030	8.50	87.20	.720	3.09
Transition	225.2	446	261	4.03	9.08	1.031	8.52	87.45	.735	3.17
Dry	212.4	712	.....	4.00	8.50	1.030	8.44	87.56	.715	3.09
Do	214.6	734	.....	4.03	8.65	1.030	8.40	87.57	.700	3.13
Transition	204.2	190	630	3.90	7.96	1.030	8.55	87.55	.725	3.11
Wet	198.6	62	810	4.13	8.20	1.030	8.47	87.40	.725	3.24
Do	192.9	88	810	4.05	7.81	1.031	8.69	87.26	.740	3.28
Average:										
Wet	215.8	92	801	4.14	8.95	.....	8.57	87.28	.732	3.20
Dry	213.5	723	.....	4.01	8.57	.....	8.42	87.56	.707	3.11

COW 24

Wet	255.1	84	774	4.10	10.50	1.035	9.56	86.34	.710	3.50
Do	251.3	72	810	4.10	10.30	1.035	9.73	86.17	.690	3.55
Transition	234.5	389	261	4.30	10.08	1.035	9.48	86.22	.690	3.51
Dry	226.4	607	.....	3.80	8.60	1.034	9.32	86.88	.645	3.37
Do	226.7	658	.....	4.00	9.07	1.033	9.21	86.79	.635	3.47
Transition	234.0	119	630	3.81	8.92	1.033	9.44	86.75	.695	3.44
Wet	237.2	56	810	4.10	9.73	1.033	9.31	86.59	.680	3.51
Do	227.1	144	810	4.10	9.31	1.034	9.51	86.39	.715	3.64
Average:										
Wet	242.7	89	801	4.10	9.96	.....	9.53	86.37	.699	3.52
Dry	226.5	632	.....	3.90	8.83	.....	9.26	86.83	.640	3.42

TABLE II.—*Comparison of the effect of turnips and a dry-roughage ration on the composition of milk*—Continued

COW 25

Ration.	Total milk.	Water in ration.	Turnips.	Fat.		Specific gravity.	Solids not fat.	Moisture.	Ash.	Total protein.
	Lb.	*Lb.*	*Lb.*	*P. ct.*	*Lb.*		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Dry	199. 8	604	.....	4. 23	8. 45	1. 033	9. 11	86. 66	. 750	3. 14
Do	195. 5	541	.....	4. 30	8. 41	1. 033	9. 07	86. 63	. 730	3. 11
Transition	218. 2	158	85	4. 25	9. 27	1. 032	8. 89	86. 86	. 745	3. 21
Wet	203. 0	.....	810	3. 98	8. 08	1. 032	9. 03	86. 99	. 715	3. 34
Do	198. 0	.....	810	4. 00	7. 92	1. 033	9. 10	86. 90	. 725	3. 40
Transition	177. 3	304	180	4. 23	7. 50	1. 032	9. 17	86. 60	. 755	3. 49
Dry	175. 3	505	.....	4. 50	7. 89	1. 032	8. 81	86. 69	. 715	3. 18
Do	160. 4	488	.....	4. 55	7. 30	1. 031	8. 87	86. 58	. 730	3. 28
Average:										
Dry	182. 7	532	.....	4. 39	8. 01	.....	9. 01	86. 64	. 731	3. 18
Wet	200. 0	.....	810	3. 99	8. 00	.....	9. 06	86. 94	. 720	3. 37

COW 27

Dry	223. 2	591	.....	4. 30	9. 60	1. 033	9. 04	86. 66	. 730	3. 13
Do	207. 7	578	.....	4. 10	8. 52	1. 033	9. 18	86. 72	. 710	3. 12
Transition	223. 3	132	585	4. 20	9. 38	1. 032	8. 90	86. 89	. 735	3. 14
Wet	237. 8	.....	810	4. 00	9. 51	1. 033	8. 81	87. 19	. 695	2. 99
Do	240. 0	.....	810	4. 04	9. 70	1. 033	9. 02	86. 94	. 765	3. 29
Transition	214. 0	407	180	4. 03	8. 62	1. 034	9. 09	86. 88	. 745	3. 24
Dry	199. 0	548	.....	4. 05	8. 06	1. 032	9. 01	86. 94	. 750	3. 28
Do	175. 4	588	.....	4. 30	7. 54	1. 031	8. 82	86. 88	. 765	3. 15
Average:										
Dry	201. 3	576	.....	4. 19	8. 43	.....	9. 01	86. 80	. 741	3. 17
Wet	238. 9	.....	810	4. 02	9. 60	.....	8. 91	87. 06	. 730	3. 14

In this series of experiments the data show conflicting results. All the cows gave more milk when fed the turnip ration, and they also ate that ration much more readily than they did the entire dry-roughage one. The two cows that were fed the ration in the order wet-dry-wet gave milk of a higher fat content on the wet ration, while those fed in the dry-wet-dry order gave the higher percentage of fat when the dry ration alone was fed. None of the other constituents of the milk were appreciably affected, and in the case of the fat content the data are so conflicting that they seem to have been caused by some factor other than the ration.

DRY VERSUS WET BEET PULP

Two cows were used in this trial, one being fed wet, dry, and wet beet pulp in successive periods, with a transition period after each change in ration, and the ration of the second cow being just the reverse. While being fed dry beet pulp each cow received 10 pounds daily. The wet ration consisted of 40 pounds of the wet beet pulp, or 10 pounds of the

dry, with 30 pounds of water added, the pulp used having been found to absorb three times its weight of water. In all conditions except as to the pulp the two rations were alike for each cow in the different periods. In Table III the quantity of water in the beet pulp, as well as the quantity of water drunk, is given:

TABLE III.—*Comparison of the effect of dry beet pulp and wet beet pulp on the composition of milk*

COW 22

Ration.	Total milk.	Water in ration.	Pulp.	Fat.		Specific gravity.	Solids not fat.	Moisture.	Ash.	Total protein.
	Lb.	*Lb.*	*Lb.*	*Per ct.*	*Lb.*		*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Dry	209. 6	590	.....	4. 80	10. 06	1. 034	9. 93	85. 27	0. 769	3. 65
Do	201. 8	540	.....	4. 85	9. 79	1. 036	10. 08	85. 07	. 797	3. 70
Wet	199. 3	273	300	4. 65	9. 27	1. 035	10. 00	85. 35	. 789	3. 89
Do	189. 5	306	300	4. 65	8. 81	1. 035	10. 15	85. 20	. 796	3. 88
Transition	185. 4	487	49	4. 80	8. 90	1. 035	9. 85	85. 35	. 795	3. 92
Dry	180. 9	479	.....	4. 80	8. 68	1. 036	9. 76	85. 44	. 797	3. 88
Do	167. 7	472	.....	4. 90	8. 22	1. 035	9. 59	85. 51	. 790	3. 82
Average:										
Dry	190. 0	520	.....	4. 84	9. 19	.....	9. 84	85. 32	. 788	3. 76
Wet	194. 4	290	300	4. 65	9. 04	.....	10. 07	85. 27	. 792	3. 88

COW 18

Wet	193. 7	340	300	5. 10	9. 88	1. 032	9. 23	85. 67	. 740	3. 11
Do	185. 0	369	300	5. 20	9. 62	1. 033	9. 19	85. 61	. 747	3. 30
Dry	196. 6	472	.....	5. 00	9. 83	1. 034	9. 60	85. 40	. 730	3. 51
Do	176. 9	511	.....	5. 40	9. 55	1. 033	9. 48	85. 12	. 733	3. 41
Transition	183. 2	348	228	5. 20	9. 53	1. 034	9. 45	85. 35	. 760	3. 62
Wet	166. 9	327	300	5. 60	9. 35	1. 035	9. 40	85. 00	. 766	3. 64
Do	159. 6	383	300	5. 60	8. 94	1. 034	9. 30	85. 10	. 754	3. 65
Average:										
Wet	176. 3	355	300	5. 37	9. 45	.....	9. 28	85. 34	. 752	3. 42
Dry	186. 7	492	.....	5. 20	9. 69	.....	9. 54	85. 27	. 731	3. 46

The data from these two cows give negative results so far as the effect of the water in the ration upon the composition of the milk is concerned. One cow, No. 22, gave milk slightly lower in fat content when the wet beet pulp was fed; but the other gave opposite results, the milk testing higher than that produced when the preceding dry ration was fed. The quantity of milk produced by both cows decreased at a normal rate.

GREEN VERSUS CURED CRIMSON CLOVER

In this series of experiments four cows were used. For a period of 10 days they were each fed all the fresh-cut green crimson clover that they would consume, and composite samples were taken during the period.

Later, when the clover had been harvested and had become well cured, the same four cows were fed all the cured product that they would consume, and composite samples again taken. No weights of water drunk were taken, but as the green clover contained 71.23 per cent of water and the cured hay but 8.33 per cent, there was an appreciable difference in the quantity of water in the rations of the two test periods. Table IV gives the results for each cow. The figures in parentheses following the class of ration show the total number of pounds of the cured or green clover fed.

TABLE IV.—*Comparison of the effect of green and cured crimson clover on the composition of milk*

Ration.	Milk.	Total water in roughage.	Fat.		Specific gravity.	Moisture.	Ash.	Total protein.
	Lb.	*Lb.*	*Per ct.*	*Lb.*		*Per ct.*	*Per ct.*	*Per ct.*
COW 23								
Green (405)	132.0	288	5.81	4.40	1.029	86.94	0.723	3.18
Cured (180)	107.1	15	4.53	4.23	1.031	86.97	.744	3.38
COW 25								
Green (415)	163.2	296	4.05	6.61	1.030	87.26	.724	3.17
Cured (180)	167.3	15	3.60	6.02	1.032	87.45	.742	3.19
COW 27								
Green (400)	161.1	285	3.75	6.04	1.030	87.58	.738	3.05
Cured (165)	128.0	14	3.60	4.61	1.032	87.35	.783	3.17
COW 201								
Green (505)	333.5	360	3.65	12.17	1.028	88.36	.696	2.78
Cured (220)	297.2	18	3.20	9.51	1.030	88.85	.725	2.77

The length of time covered by this series of experiments, 10 days on each ration, was too short to give more than an indication of the results which a complete investigation would give. The data obtained, however, show that the water in the ration supplied by a green roughage, as compared with the cured product, does not lower the fat content of the milk. The results of these experiments would even indicate an opposite effect, for in all cases the cows gave higher testing milk and three of them produced more milk on the green feed.

SUMMARY

Four different methods of varying the water content of the ration were used in this work.

(1) A full versus a limited allowance of drinking water.
(2) Turnips versus a dry-roughage ration.
(3) Wet versus dry beet pulp.
(4) Green versus dry crimson clover.

Eight cows were used in the experiments conducted by the first method, four in the second, two in the third, and four in the fourth.

In every case except when the crimson clover was fed the amount of water drunk by the different animals, as well as the difference in the water content of the roughages under comparison, was determined.

With all except one cow, No. 22 in the wet versus dry beet-pulp group, the amount of water in the dry ration did not exceed 75 per cent of that supplied by the wet ration, and with some cows that were given a limited allowance of water the dry ration contained less than 60 per cent of the water content of the full-allowance ration.

Cow 22 in the wet versus dry beet-pulp group received, when the dry ration was fed, 88 per cent of the water content of the wet ration.

In the green versus cured crimson-clover group, the former contained 71.23 per cent water and the latter 8.33 per cent. The daily ration of green clover varied from 40 to 50 pounds per head, and of the cured hay from 16 to 22 pounds per head.

Certain individual cows at times produced milk having an abnormal fat content. This effect was apparently independent of the ration, as it occurred not only with the high water-content ration but with the dry as well. A study of the data obtained in the four series shows that the watery character of the ration has no effect upon the fat content of the milk.

There was even less variation in the other milk constituents than in the fat. This indicates that rations of varying water content have no effect upon the composition of milk.

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CROWNGALL STUDIES SHOWING CHANGES IN PLANT STRUCTURES DUE TO A CHANGED STIMULUS

[PRELIMINARY PAPER]

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Some recent experiments with crowngall have led to a number of discoveries which may be summarized as follows:

First, as everyone knows, the tendency of cambium is not simply to go on indefinitely producing more cambium but to elaborate out of its embryonic elements formed structures, tracheids, wood vessels, wood fibers, ray cells, sieve tubes, etc., all having a definite arrangement and a well-defined polarity, but when internodal-stem cambium is inoculated with the crowngall organism (*Bacterium tumefaciens*) the ordinary physiological tendencies are upset, as already shown in 1911 and 1912,[1] and several very interesting new phenomena make their appearance: (1) The elements of the formed or mature tissues are produced in less numbers than ordinarily, and these elements have lost the whole or a considerable part of their polarity, so that the most bizarre complexes of twisted and distorted tissues arise; (2) the parenchymatous elements are greatly increased in number and reduced in size, since under the bacterial stimulus many of the cambium cells appear to have lost all power to produce mature tissues and at the same time have acquired a new growth impetus, a tendency to an uncontrolled, pathologically embryonic, cell multiplication, the result of which is a tumor of indefinite extension—the ordinary naked crowngall, containing the distorted formed elements above referred to and in addition exhibiting a marked hyperplasia of the parenchyma; (3) correlative with these changes, over which the plant has no control, is a tendency to open wounds and to early decay and also to the formation of daughter tumors.

Second, when, by means of very shallow needle pricks, similar inoculations are made into the internodal cortex of young stems (the so-called fundamental tissue, which is still in a growing condition) a similar cell proliferation occurs, the elements of which are very small in comparison with those from which they have developed, because under the changed stimulus they are kept embryonic and are compelled to divide soon after previous divisions, so that they can never reach maturity either in size

[1] Smith, Erwin F., Brown, Nellie A., and Townsend, C. O. Crown-gall of plants: its cause and remedy. U. S. Dept. Agr. Bur. Plant Indus. Bul. 223, 215 p., 36 pl. 1911.

Smith, Erwin F., Brown, Nellie A., and McCulloch, Lucia. The structure and development of crown-gall: a plant cancer. U. S. Dept. Agr. Bur. Plant Indus. Bul 255, 60 p., 109 pl. 1912.

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or function as long as the stimulus lasts. These inoculations (on the Paris daisy) have brought out another interesting fact. As the tendency of young fundamental tissue (the growing point) is to form a stele in its center, so when the mature tissues of the stem cortex are brought under the new stimulus and begin to proliferate, in the manner of embryonic tissues, primitive but imperfect stele-forming tendencies are developed in the tumor. I have not seen an actual shoot produced by such a tumor; but sieve tubes and trachei are formed in it (out of descendants of cortex cells, be it remembered); and cross sections of some of these small tumors show that these stelar elements have a tendency to be arranged in the form of a closed structure (primitive stele), although often this is not pronounced. These superficial tumors have no connection with the xylem or phloem of the true stele, for in no case did the needle punctures enter as far as the phloem, much less the cambium, and serial sections show clearly that all of their structures (blastomous cells, trachei, and sieve tubes) have been developed wholly, out of cortex cells (probably cortex mother cells). After a few weeks such shallow tumors cease to grow, or develop very slowly, owing to imperfect nutrition (lack of all connection with the xylem and phloem of the plant).

Third, when the crowngall organism (hop strain) is inoculated into the leaf axils of young growing plants (species of Pelargonium, Nicotiana, Lycopersicum, Citrus, Ricinus, etc.) the buds of which are in a dormant state and which under ordinary conditions will continue dormant—namely, unless the top of the plant is removed—a new type of tumor develops, one hitherto not seen in crowngall. Inoculating in this way I have obtained tumors covered all over with diminutive, abortive leafy shoots, or flower shoots, if flower anlage have been disturbed. The shoots may be variously twisted, fused, and fasciated, as in the common house geranium (*Pelargonium* spp.) shown in Plate XVIII. This apparently is what happens: The growth of the tumor distorts the tissues, tearing the anlage into small fragments which are variously distributed and develop on or in the tumor into organs of a size proportional to the size of the included fragment—here as part of an ovary or anther, there as a shoot. These pathological shoots live but a short time and are quite unable to carry on the normal activities of the plant when the other leaves are removed. I have believed for a long time that fasciation must be due to a bacterial infection; but this is, I believe, the first time that anyone has obtained it by means of a pure-culture inoculation.

The results obtained by inoculating the upper leaf axils of young growing plants of the castor-oil plant (*Ricinus communis*) are prompt and quite as striking (Pl. XIX).

On tobacco plants (*Nicotiana tabacum*) these teratoid tumors, developed in leaf axils (Pls. XX and XXII), have also produced secondary tumors repeating the structure of the parent tumor. Such tumors have been obtained both in stems and leaves, especially when inoculations were

made early; and they contain, along with the proliferating tumor cells (blastomous cells), the same teratoid elements as the primary tumor. These are true daughter tumors, being connected back to the primary tumor by a tumor strand which is quite different both in structure and in location (Pl. XXI) from that occurring in the Paris daisy. The latter, it will be remembered, follows the line of the spiral vessels in the inner wood, and is parenchymatous in its structure, containing only here and there some vessels (scattered trachei). This tobacco tumor strand occurs in the cortex, consists almost entirely of vessels, and is a true stem (stele), although developed under a pathological stimulus, and in a part of the plant where no stele was ever seen before—namely, in the outer cortex, through which it can be traced (parallel to the long axis of the stem) for long distances and from which at intervals leafy tumors are sent to the surface of the plant. From its frequent proliferation in the form of tumors it is evident that parenchymatous (blastomous) elements must also occur in the strand, but they are not abundant. In fact, in the parts I have examined they are almost as infrequent as are trachei in the daisy strand. Cross sections and longitudinal sections of this remarkable tumor strand show it to have spiral vessels in its center, surrounded by trachei cut by ray cells, beyond which is a cylinder of cambium surrounded by a cylinder of phloem, containing well-developed sieve tubes. This tiny stele has no cortex or epidermis because it does not need any, being surrounded and sufficiently protected by the normal cortex of the tobacco stem. This is a phenomenon due apparently to my new manner of inoculation (into shoot anlage), because some years ago by inoculating internodally on tobacco stems I obtained and figured[1] tumors and a tumor strand in cortex corresponding to those found in the Paris daisy—that is, composed chiefly of small-celled parenchyma. The difference in results must therefore be due to difference in the kind of tissue inoculated, each developing pathologically according to its own growth tendencies.

Fourth, on some plants (which were tobaccos) I have also obtained leafy tumors by making my bacterial inoculations *in places where no bud anlage are known to exist*—for example, in the middle of leaves. Ordinarily when leaf tissue in tobacco grows, it only produces more leaf tissue;[2] but when the crowngall organism (hop strain) is pricked into midribs or side veins, tumors arise and a portion of them are leafy—that is, bear shoots. I have obtained 27 such leafy tumors on a single plant and several on a single leaf, all within a period of a few weeks (Pl. XXIII). It is easy to obtain them. The young leaves yield a larger proportion of such tumors than the older ones, and I have observed no shoot-bearing tumors on leaves which were fairly well developed when inoculated.

[1] Smith, Erwin F., Brown, Nellie A., and McCulloch, Lucia. The structure and development of crowngall: a plant cancer. U. S. Dept. Agr. Bur. Plant Indus. Bul. 255, pl. 102-103. 1912.

[2] I have never got any leaf cuttings of it to take root.

Rapidly developing young tissues seem to be necessary. Here again, a changed stimulus has produced a more embryonic and primitive condition, as shown by the appearance of these shoots. It is a pathological phenomenon, but one of more than passing interest, for, unless I am much mistaken, it has wide physiological and pathological bearings. It is another proof that the immature cell wherever it is located carries the inheritance of the whole organism, and that what it will finally become, as it matures, depends on the stimuli withheld from it or applied to it. In other words, it is so much evidence that any young cell may become a totipotent cell if it is subjected to the proper stimulus, and this stimulus may be either *physiological*, resulting in a normal structure, as when the top of a plant is removed and a new top grows in its place out of so-called adventitious buds (regeneration phenomena), or *pathological*, resulting in an embryonic teratoma, as when a tumor-producing schizomycete is introduced into sensitive growing tissues.

PLATE XVIII

Teratoid crowngalls produced in *Pelargonium* spp. by inoculating *Bacterium tumefaciens* (hop organism through sunflower) into upper leaf axils on January 13, 1916. Photographed at the end of 74 days. At *X* the top of the shoot bearing five or six leaves was removed to show the tumor more distinctly. All of the leafy shoots here shown and many others too small to be seen distinctly in the photograph are outgrowths from the tumor, which also bears red abortive flower anlage. The upper shoot (*L*) was also flattened and fasciated (several shoots fused together) and the front leaves (*P P*) were turning yellow and dying.

L
P
P
X

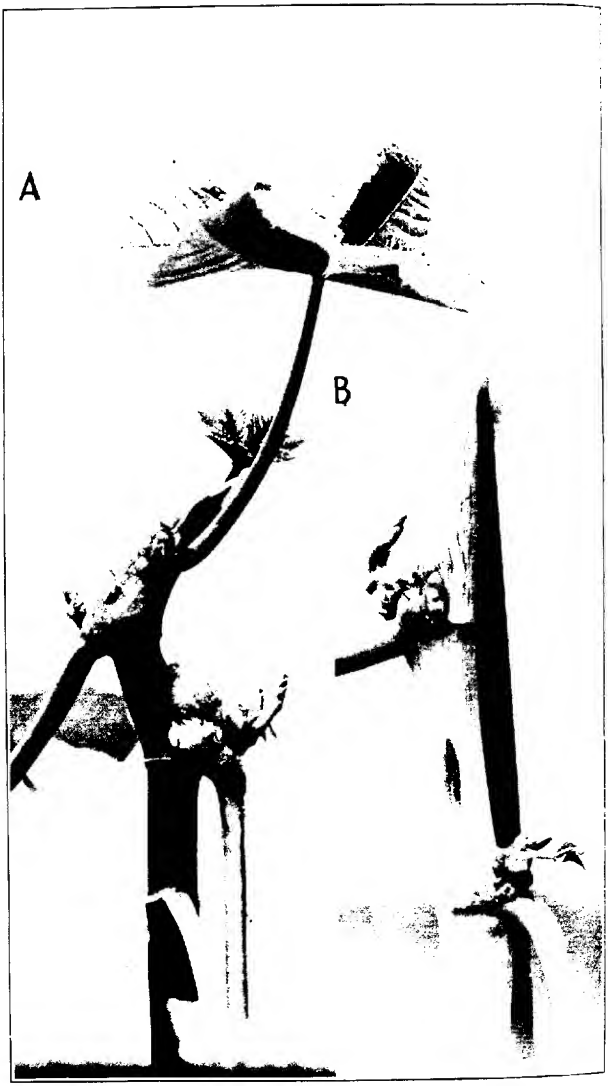

A
B

PLATE XIX

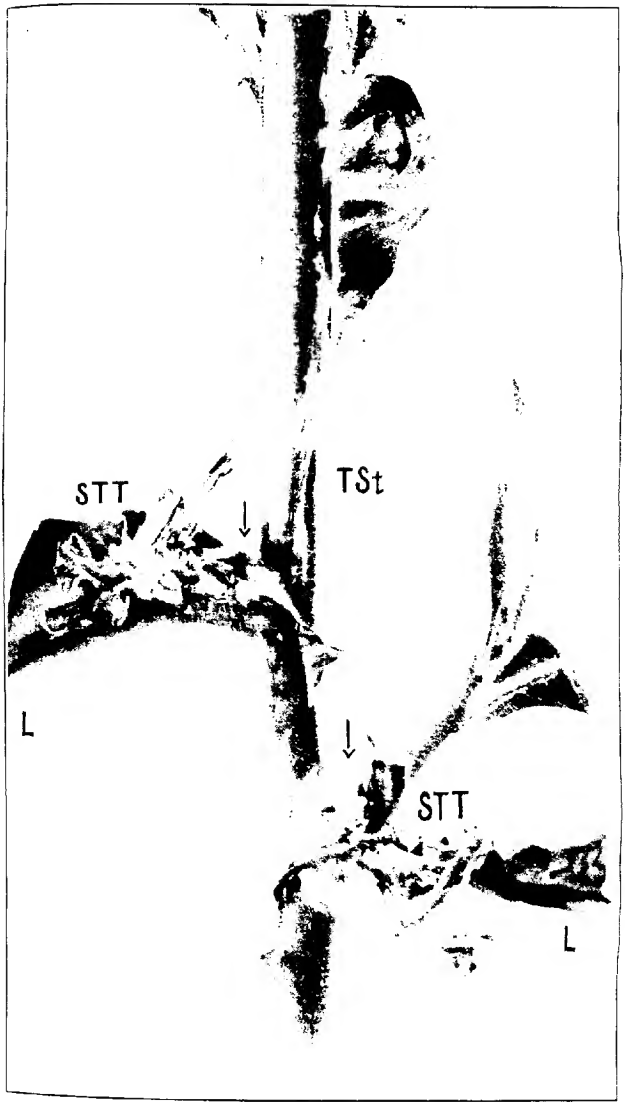
Teratoid crowngalls produced in castor-oil plant (*Ricinus communis*) by inoculating *Bacterium tumefaciens* (hop strain), the inoculations being made in the upper leaf axils of young, vigorous, unbranched plants.

Fig. A.—A red-stem variety. Leaves reflexed; axis distorted; and feeble shoots developing out of the axillary tumors. There are on the tumors other smaller shoots not shown here distinctly. Time, 13 days.

Fig. B.—A green-stem glaucous variety. As in figure A, but time 17 days. Here also internal growths (root anlage) are pushing up the tissues of the stem below the lower leaf. A few days later these roots appeared on the surface, both of this internode and of the one above it. This phenomenon has been recorded previously by the writer as sometimes occurring on inoculated stems of the Paris daisy and other plants in the vicinity of developing tumors (Smith, E. F. Bacteria in Relation to Plant Diseases, vol. 2, fig. 26. 1911).

PLATE XX

Teratoid crowngalls produced in tobacco by inoculating *Bacterium tumefaciens* (isolated from a hop tumor several years ago and passed through a sunflower in 1915). The inoculations were made by needle pricks in the axils of the lower leaves (under the arrows), at which places small leafy tumors developed. These sent tumor strands into the midribs of both leaves (*L L*) and later secondary teratoid tumors (*S T T*) burst through and covered the top of the midrib. From the upper leaf axil also a tumor strand developed, passing upward through 5 internodes and then out into the midrib of a leaf for several inches, giving rise at frequent intervals to tumors bearing leafy shoots (teratoid elements) and to others free from them. This tumor strand (*T St*) was not on the surface of the stem, as might appear from the photograph, but was near enough to show through as a translucent band about 2 mm. wide. Time, 26 days.


STT
TSt
L
STT
L

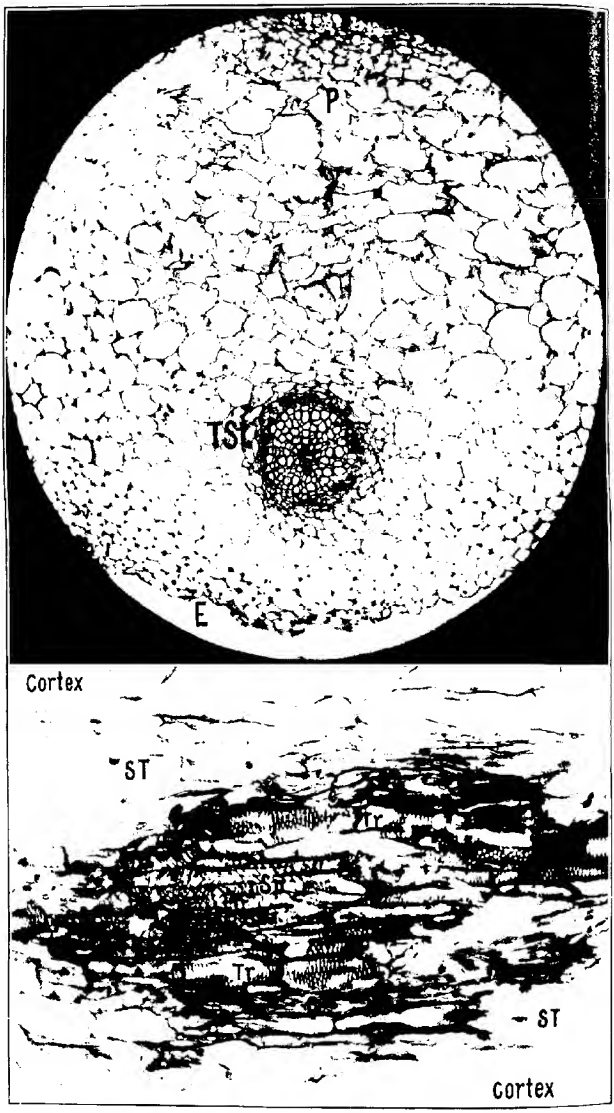

P
TST
E
Cortex
ST
Tr
TST
Tr
ST
Cortex

PLATE XXI

The teratoid tumor strand of Plate XX, which gives rise during its course to more than 30 small tumors.

Top. Cross section of outer part of right side of stem of tobacco plant shown on Plate XX. *P*, outer edge of the phloem; *E*, epidermis; *T St*, tumor strand, which is bedded in the normal cortex of the stem.

Bottom. Longitudinal section from upper part of the above tumor strand, more highly magnified, showing it to be a true stele. The coarse-celled tissue at top and bottom is the normal cortex of the stem. The pathological tissues are *S T*, sieve tubes; *C*, cambium; *Tr*, trachei; *Sp*, spiral vessels.

PLATE XXII

Teratoid crowngalls produced in a tobacco plant by inoculating *Bacterium tumefaciens* (hop strain through sunflower) into the leaf axils. Small tumors soon appeared where inoculated and these are now covered with pale leafy shoots which have swollen (tumefied) bases and are beginning to die. The top was cut away on the 26th day, and the plant was unable to make a new one out of these pathological shoots, but has grown it (*X*) from an uninoculated lower leaf axil. Time, 73 days.

X

A
C
B

PLATE XXIII

Teratoid crowngalls produced in tobacco leaves with the hop strain of *Bacterium tumefaciens* by local (leaf) inoculations—that is, inoculation in places where shoot anlage are not known to exist.

Fig. A.—Portion of an upper leaf showing four shoot-bearing tumors growing from upper surface of the inoculated midrib. Leaf inoculated February 16, 1916. Photographed on April 1.

Fig. B.—Same as *A*, but the leaf reversed and the midrib stripped of its blade to show two other shoot-bearing tumors which have developed from its under surface. Actual height of the tallest shoot, 1.5 cm.

Fig. C.—From middle of another leaf on the same plant as *A*, but further magnified and photo made on an orthochromatic plate to show the pale green character of the shoot as contrasted with the dark green of the surrounding leaf (which is also in shadow). This tumor and its shoot arise from a branch of the midrib, the latter in cross section being shown at *X*. A smaller teratoid tumor bearing two shoots (at either side of *C*) developed on the upper surface of the leaf and the one bearing the longer shoot on its lower surface. The actual length of this shoot was 1.5 cm. The leaf was curved downward and the shoot was growing out horizontally. Time, 45 days.

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